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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Design, synthesis and QSAR study of arylidene indoles as anti-platelet aggregation inhibitors

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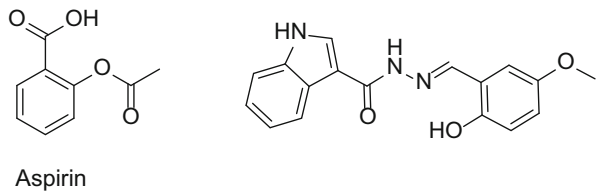
Abstract A series of novel substituted indole carbohydrazide was synthesized and evaluated for anti-platelet aggregation activity. The structures of the synthesized compounds were confirmed by spectral data and elemental analysis and were evaluated for their ability to inhibit platelet aggregation induced by adenosine diphosphate, arachidonic acid (AA) and collagen. Compounds **3e** and **3b** exhibited the highest activities against the platelet aggregation induced by collagen with IC_{50} values of 12.7 and 13.3 μM, respectively, and **2h** with IC_{50} value of 51.88 μM and **2i** with IC_{50} of 44.38 μM efficiently inhibited platelet aggregation induced by AA. The QSAR investigation indicated the importance of the topological, constitutional and geometrical parameters (PW3, PW4, LP1 and GATS6v) in describing the anti-platelet aggregation activity of the synthesized hydrazides. Evaluation of cytotoxic activity of the compounds against L929 cell line and three cancer cell lines revealed that none of the compounds have significant cytotoxicity.

Graphical Abstract



Keywords Anti-platelet aggregation · Cytotoxicity · Indole · *N*-acylhydrazone · QSAR

Introduction

A primary factor in the development of thrombotic disorders, such as unstable angina, myocardial infarction, stroke and peripheral vascular diseases, is platelet aggregation which plays a central role in thrombosis (clot formation) (Reddy *et al*., 2011).

Blood is not aggregated in the blood vessels, but when bleeding occurs, blood aggregation is occurred as a physiological defense reaction. Activation of the platelets by a complex of biochemical pathways and multitude of mediators is the primary step in the progress of these diseases (Mashayekhi *et al*., 2013a, b). However, platelet aggregation is also caused by physiological substances, such as thrombin and prostaglandin endoperoxide, and it leads to an arterial thrombosis. Platelets can be activated by a number of endogenous agonists such as arachidonic acid (AA), adenosine 5′-diphosphate (ADP), thromboxane A_2 (TxA_2), thrombin, platelet activating factor (PAF),

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epinephrine (EPN) and collagen (DeCandia *et al*., 2009; Reddy *et al*., 2011).

Acetyl salicylic acid (a non-selective irreversible COX inhibitor) and clopidogrel (an irreversible antagonist of P2Y12) are the most commonly prescribed oral anti-platelet drugs to date (Mashayekhi *et al*., 2013a, b). However, definite disadvantageous effects of these compounds, for instance, a number of drug-class-specific adverse reactions such as gastrointestinal ulcers, bleeding, thrombocytopenic purpura and skin necrosis are repeatedly reported. Also, inefficient therapies and their related problems cause anti-platelet therapy more complicated.

Therefore, many research groups have made significant attempts to modify the potency and platelet anti-aggregatory activity of common drugs (Siwek *et al*., 2011).

N-acylheteroarylhydrazones (NAH) have been recently reported as platelet aggregation inhibitors. Bezerra-Neto *et al*., (2006) have previously described the *N*-acylhydrazone subunit as a pharmacophore group for analgesic, anti-inflammatory and anti-platelet properties, and it is also considered a privileged structure in the design of new bioactive compounds (Duarte *et al*., 2007; Brito *et al*., 2010). Coquelet et al. have reported some acylhydrazone derivatives as inhibitors of platelet cyclooxygenase (COX) and lipooxigenase (LOX) blocking the transformation of arachidonic acid to its proaggregatory metabolites. In light of the finding of this study, Barreiro et al., in a series of studies, managed to develop some new hydrazones which effectively inhibited platelet aggregation with selective inhibitory activity toward platelet aggregation induced by arachidonic acid. They found active group of hydrazones with arylsulfonate acylhydrazone, phenothiazine-1-acylhydrazone, *N*-substituted-phenyl-1,2,3-triazole-4-acylhydrazone and pyrazolylhydrazone structures. Although a diverse group of derivatives have been found to exhibit anti-platelet activity in these studies, they share a preserved structural backbone that is two (hetero)aromatic ring systems linked by a hydrazone bond (Haj Mohammad Ebrahim Tehrani *et al*., 2013) (Fig. 1).

Coquelet et al. reported the most active derivatives with R = CH3 and NH2, while Barreiro et al. have mainly focused on the derivatives with R = H. Cunha *et al*., (2003) also synthesized *N*-phenylpyrazolyl moiety which has the NAH chain that exhibited inhibitory effect on AA-, collagen- and ADP-induced platelet aggregation so, represented a novel family of anti-platelet agents. Silva et al. suggest that the hydrazone and acylhydrazone moieties demonstrate a subunit which stabilize free radicals that mimicking bis-allyl fragment of unsaturated fatty acids such as arachidonic acid. Furthermore, these fragments have an important role as pharmacophore cores with anti-inflammatory, anti-nociceptive and anti-platelet aggregation activity (Silva *et al*., 2004).

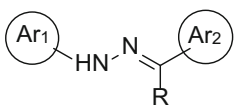


Fig. 1 Schematic representation of the general hydrazone structural backbone with anti-platelet activity

Nazare *et al*., (2004) introduced indolecarboxamides as a rigid α-amino acid mimetic and very flexible moiety, enabling to represent different structures at its nitrogen or also carboxy group (Nazare *et al*., 2004). Moreover, in a successful study, indole ring is demonstrated as a structural moiety which has anti-platelet aggregation effect. Indole-3-carbinol, a natural compound found in cruciferous vegetables, is known to have anti-platelet and anti-thrombotic activities in vitro and in vivo. Indole-3-carbinol has been shown to inhibit collagen-induced platelet aggregation in human platelet-rich plasma (PRP) in dose-dependent manner (Mashayekhi *et al*., 2013a, b; Park *et al*., 2008).

In our ongoing research addressed toward new anti-platelet aggregation agents which showed inhibitory effects on AA-, collagen- and ADP-induced aggregation, a series of indole hydrazone compounds have been designed and synthesized by molecular hybridization between indole and hydrazones moieties, as potential anti-platelet aggregation derivatives (Haj Mohammad Ebrahim Tehrani *et al*., 2013; Mashayekhi *et al*., 2013a, b). In order to investigate the potential correlation between activity and positional isomerism in the derivatives, two groups of compounds were synthesized with various B-ring substituents on position 2 and position 3 of indole ring, and the compounds were screened for their inhibitory effects on platelet aggregation, induced by arachidonic acid, ADP and collagen. Thus, in this research, we report the synthesis, platelet aggregation inhibitory effects and SAR study of new indole acylhydrazone derivatives (Fig. 2).

Materials and methods

General

All commercial solvents, chemicals and reagents were purchased from either Merck or Sigma-Aldrich with the highest purity and used without further purification. Proton nuclear magnetic resonance (^{1}H NMR) spectra were

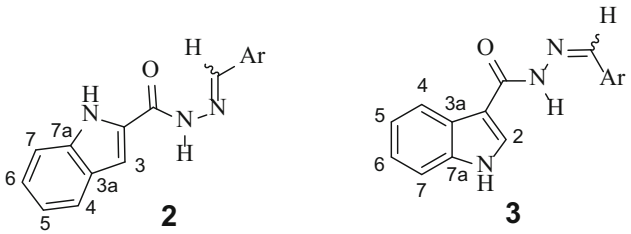


Fig. 2 Main scaffolds of target compounds

Table 1 In vitro anti-platelet aggregation activity of the synthesized compounds (2a-m and 3a-r) induced by AA, ADP and collagen

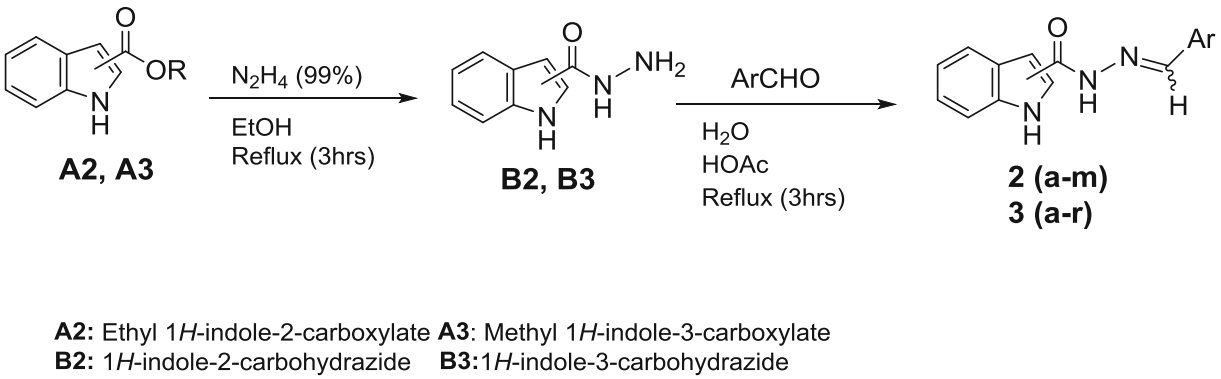
Comp.	Ar	IC_{50}(μM)[a]		Inhibition (%)[b]
		Collagen	AA	ADP
2a	Phenyl	134 ± 4.7	94 ± 2.5	50.6 ± 2.8
2b	2,4-Dichlorophenyl	796 ± 19.9	880 ± 8.9	47.6 ± 0.8
2c	2,6-Dichlorophenyl	705 ± 21.1	837 ± 4.6	21.5 ± 1.5
2d	4-Methoxyphenyl	>1000	142 ± 1.8	66.7 ± 3.0
2e	2-Nitro-5-thiophene	328 ± 2.1	731 ± 4.7	0
2f	2-Hydroxy-5-ethoxyphenyl	>1000	>1000	0
2g	2,4-Dihydroxyphenyl	416.5 ± 12.4	414 ± 5.2	53.6 ± 2.0
2h	2,5-Dihydroxyphenyl	173.2 ± 10.4	51.88 ± 0.8	57.2 ± 1.8
2i	2,3-Dihydroxyphenyl	239.4 ± 6.3	44.38 ± 0.9	58.6 ± 0.7
2j	5-Chloro-2-nitrophenyl	>1000	>1000	58.7 ± 2.3
2k	2-Nitro-5-furane	>1000	618 ± 4.6	37.7 ± 0.6
2l	5-Bromo-2-hydroxyphenyl	520.1 ± 17.7	>1000	61.2 ± 3.3
2m	3-Methoxyphenyl	446.7 ± 15.1	137 ± 2.1	20.6 ± 1.0
3a	Phenyl	20.9 ± 1.4	182.6 ± 6.1	80.3 ± 1.0
3b	2,6-Dichlorophenyl	13.3 ± 1.0	251.2 ± 5.8	71.24 ± 0.8
3c	2,4-Dichlorophenyl	Not dissolved	Not dissolved	Not dissolved
3d	4-Methoxyphenyl	19.5 ± 1.2	497 ± 3.9	83 ± 1.3
3e	2-Hydroxy-5-methoxyphenyl	12.7 ± 0.9	219.8 ± 1.9	55 ± 0.5
3f	4-Nitrophenyl	23.3 ± 1.1	292 ± 2.7	50 ± 1.1
3g	3-Indole	106.8 ± 4.9	326 ± 8.0	77 ± 0.9
3h	2-Thiophene	70 ± 1.4	190 ± 1.5	80 ± 1.4
3i	5-Chloro-2-nitrophenyl	20.3 ± 1.4	292.5 ± 3.4	70 ± 0.7
3j	2,3-Dihydroxyphenyl	66.8 ± 2.6	169.8 ± 2.2	64.7 ± 2.3
3k	2,5-Dihydroxyphenyl	31.5 ± 0.8	102.5 ± 6.7	98 ± 2.6
3l	2,4-Dihydroxyphenyl	64.7 ± 2.8	229 ± 1.1	75.2 ± 3.1
3m	2,3-Dimethoxyphenyl	124.8 ± 3.5	237 ± 0.8	18 ± 1.4
3n	3,4,5-Trimethoxyphenyl	227.9 ± 9.1	530 ± 1.9	10 ± 0.4
3o	3-Nitrophenyl	95.6 ± 3.0	307.3 ± 5.0	77.8 ± 2.6
3p	2-Nitro-5-thiophene	Not dissolved	Not dissolved	Not dissolved
3q	5-Nitrofuran	Not dissolved	Not dissolved	Not dissolved
3r	3-Methoxyphenyl	295 ± 5.9	346 ± 1.7	35.7 ± 1.9
Indomethacin[c]		1.2 ± 0.1	3 ± 0.2	42 ± 1.1
Aspirin[c]		9.7 ± 0.6	30 ± 2.6	21 ± 0.6

[a] Values are presented as mean ± SD of three separate determination

[b] Inhibition (%) values for ADP as a platelet aggregation inducer represent at 1 mM concentration for (**2a–m**) and at 250 μM concentration for (**3a–r**)

[c] Aspirin and indomethacin were used as a positive control

recorded on a Bruker 500 MHz spectrometers (Bruker, Rheinstetten, Germany), pick positions are illustrated in parts per million (δ) in DMSO-d_6 solution and tetramethylsilane (0.05 % v/v) as internal standard, and coupling constant values (J) are given in Hertz. Signal multiplicities are reported by: s (singlet), d (doublet), t (triplet), q (quadruplet), m (multiplet) and brs (broad signal). For NMR spectral data assignments, the atom numbering of compounds is depicted in Table 1. Analytical thin-layer chromatography (TLC) was performed with Merck silica gel plates and visualized with UV irradiation (254 nm) or iodine. Electrospray ionization mass spectra (ESI–MS) were obtained using Agilent 6410 Triple Quad. LC/MS. Melting points were obtained by an Electrothermal 9100 apparatus and are uncorrected. The IR spectra were taken by a PerkinElmer 843 spectrometer with KBr as diluent.



Scheme 1 The synthesis pathway for indole *N*-acylhydrazones

The elemental analysis for C, H and N was performed by a Costech model 4010, and the percentage values agreed with the proposed structures within ±0.4 % of the theoretical values. All described products showed 1H NMR spectra according to the assigned structures.

Chemistry

The synthetic procedure planned to obtain the desired indole *N*-acylhydrazone derivatives is shown in Scheme 1. The key intermediates were obtained by hydrazinolysis of methyl 1*H*-indole-3-carboxylate and ethyl 1*H*-indole-2-carboxylate in 96 and 91 % yield, respectively, using hydrazine monohydrate 99 % in ethanol. The final indole *N*-acylhydrazone derivatives were obtained by condensing the hydrazide intermediates with the proper aromatic aldehydes (W-ArCHO) in water and glacial acetic acid as the solvent, in good yields.

The derivatives were obtained by the reaction of 1*H*-indole-3-carbohydrazide (**B3**) and 1*H*-indole-2-carbohydrazide (**B2**) with the proper aldehydes. Synthesis of Schiff bases was performed in ethanol with a few drops of glacial acetic acid. This reaction in the majority of the cases was straightforward, but the products were soluble in ethanol and their separation was difficult. Thus, water was used as solvent and a few drops of glacial acetic acid were added to the reaction mixture and heated to complete the reaction.

*General procedure for the preparation of carbohydrazides (**B2** and **B3**)*

1*H*-indole-3-carbohydrazide (**B3**) and 1*H*-indole-2-carbohydrazide (**B2**) were prepared according to the literature method (Mirfazli *et al*., 2014, Bao *et al*., 2013). Compounds **2b**, **2j** and **2k** in which *E*/*Z* ratio was higher than 10 percent were distinguished through their ^{1}H and ^{13}C NMR spectra.

*General procedure for the preparation of N-acylhydrazone derivatives (**2a–m**) and (**3a–r**)* Titled compounds (*N*-acylhydrazone derivatives) were obtained according to the literature method (Mirfazli *et al*., 2014).

*N′-benzylidene-1H-indole-3-carbohydrazide (**3a**)* The derivative **3a** was obtained as a white solid by condensation of **B3** with benzaldehyde in 82.5 % yield, mp 232–234 °C. IR (KBr) cm^{-1}: 3282, 3206, 3115, 3059, 3032, 1629, 1601, 1576, 1547; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.80 (brs, 1H, Indole-NH), 11.44 (s, 1H, CONH), 8.31 (bs, 1H, –N=CH–), 8.21–8.42 (m, 2H, Indole H_2, H_4), 7.72 (d, 2H, J = 6.8 Hz, –N=CH–C_6H_5 H_2, H_6), 7.41–7.49 (m, 4H, Indole H_7, –N=CH–C_6H_5 H_3, H_4, H_5), 7.18 (m, 2H, Indole H_5, H_6); ^{13}C NMR (125 MHz, DMSO-d_6) δ, 161.25 (C=O), 144.72 (C=N), 142.55 (C3a), 135.95 (C1′ benzyl), 134.76 (C4′ benzyl), 134.52 (C2), 132.22 (C7a), 129.47 (C2′ and C6′ benzyl), 129.19 (C3′ and C5′ benzyl), 128.78 (C4), 122.21 (C6), 121.16 (C5), 111.94 (C3), 108.50 (C7). ESI-Mass *m/z*: 264 $[M + H]^+$, 286 $[M + Na]^+$, 302 $[M + K]^+$; Anal. Calcd. for $C_{16}H_{13}N_3O$: C, 72.99; H, 4.98; N, 15.96. Found: C, 72.62; H, 5.08; N, 15.72 (Alemany *et al*., 1966).

*N′-(2,6-dichlorobenzylidene)-1H-indole-3-carbohydrazide (**3b**)* The derivative **3b** was obtained as a white solid by condensation of **B3** with 2,6-dichlorobenzaldehyde in 86 % yield, mp 281–283 °C. IR (KBr) cm^{-1}: 3412, 3234, 3180, 3086, 1651, 1614, 1592, 1556; ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.79 (bs, 1H, Indole-NH), 11.40 (s, 1H, CONH), 8.48 (bs, 1H, –N=CH–), 8.35 (bs, 1H, Indole H_2), 8.24 (d, 1H, J = 8.0 Hz, Indole H_4), 7.58 (d, 2H, J = 8 Hz, –N=CH–C_6H_5, H_3, H_5), 7.48 (d, 1H, J = 8.0 Hz, Indole H_7), 7.43 (t, 1H, J = 8 Hz, –N=CH–C_6H_5 H_4), 7.22–7.15 (m, 2H, Indole H_5, H_6). ^{13}C NMR (125 MHz, DMSO-d_6): δ 153.25 (C=O), 139.57 (C=N), 137.81 (C3a), 135.77 (C2′ and C6′ 2,6-dichlorophenyl), 133.76 (C2), 130.78 (C1′ 2,6-dichlorophenyl), 129.13 (C4′

2,6-dichlorophenyl), 126.84 (C7a),123.09 (C3′ and C5′ 2,6-dichlorophenyl), 122.25 (C4), 121.16 (C6), 120.85 (C5), 111.91 (C3), 107.48 (C7). ESI-Mass *m/z*: 354 $[M + Na]^+$; Anal. Calcd. for $C_{16}H_{11}Cl_2N_3O$: C, 57.85; H, 3.34; N, 12.65. Found: C, 57.63; H, 3.48; N, 13.02.

*N′-(2,4-dichlorobenzylidene)-1H-indole-3-carbohydrazide (**3c**)* The derivative **3c** was obtained as a white solid by condensation of **B3** with 2,4-dichlorobenzaldehyde in 78 % yield, mp 288–291 °C. IR (KBr) cm^{-1}: 3472, 3316, 3282, 3126, 3103, 1659, 1612, 1594,1569.1540; ^1H NMR (500 MHz, DMSO-d_6): δ 11.79 (s, 1H, Indole-NH), 11.64 (s, 1H, CONH), 8.66 (bs, 1H, –N=C<u>H</u>–), 8.25 (bs, 1H, Indole H_2), 8.19 (d, 1H, $J = 7.6$ Hz, Indole H_4), 8.02 (d, 1H, $J = 8.5$ Hz, –N=CH–C_6<u>H_5</u>, H_6), 7.71 (d, 1H, $J = 2.25$ Hz, –N=CH–C_6<u>H_5</u>, H_3), 7.53 (dd, 1H, $J = 8.55$, 2.07 Hz, –N=CH–C_6<u>H_5</u>, H_5), 7.48 (d, 1H, $J = 7.6$ Hz, Indole H_7), 7.19 (m, 2H, Indole H_5, H_6). ^{13}C NMR (125 MHz, DMSO-d_6): δ 157.15 (C=O), 139.57 (C=N), 137.20 (C3a), 135.77 (C2′ 2,4-dichlorophenyl), 133.76 (C2), 129.13 (C1′ 2,4-dichlorophenyl), 128.35 (C3′ 2,4-dichlorophenyl), 127.23 (C6′ 2,4-dichlorophenyl), 126.84 (C7a), 125.12 (C4′ 2,4-dichlorophenyl), 122.25 (C5′ 2,4-dichlorophenyl), 121.16 (C4), 120.85 (C6), 111.91 (C5), 108.01 (C3), 107.48 (C7). ESI-Mass *m/z*: 354 $[M + Na]^+$; Anal. Calcd. for $C_{16}H_{11}Cl_2N_3O$: C, 57.85; H, 3.34; N, 12.65. Found: C, 57.71; H, 3.20; N, 12.92.

*N′-(4-methoxybenzylidene)-1H-indole-3-carbohydrazide (**3d**)* The derivative **3d** was obtained as a white solid by condensation of **B3** with 4-methoxybenzaldehyde in 61 % yield, mp 239–241 °C. IR (KBr) cm^{-1}: 3386, 3300, 3163, 3137, 1667, 1629, 1594, 1558; ^1H NMR (500 MHz, DMSO-d_6): δ 11.71 (s, 1H, Indole-NH), 11.27 (s, 1H, CONH), 8.30–8.20 (m, 3H, Indole H_2, H_4, –N=C<u>H</u>–), 7.66 (d, 2H, $J = 8.70$ Hz, –N=CH–C_6<u>H_5</u>, H_2, H_6), 7.48 (d, 1H, $J = 7.85$ Hz, Indole H_7), 7.21–7.14 (m, 2H, Indole H_5, H_6), 7.03 (d, 2H, $J = 8.70$ Hz, –N=CH–C_6<u>H_5</u>, H_3, H_5), 3.82 (s, 3H, –OCH_3). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 161.11 (C=O), 160.39 (C4′ 4-methoxyphenyl), 144.75 (C=N), 144.31 (C3a), 135.93 (C2), 128.24 (C2′ and C6′ 4-methoxyphenyl), 127.33 (C7a), 126.73 (C1′ 4-methoxyphenyl), 122.15 (C4), 121.13 (C6), 120.65 (C5), 114.29 (C3′ and C5′ 4-methoxyphenyl), 111.89 (C3), 108.59 (C7), 55.25 (O-<u>C</u>H3). ESI-Mass *m/z*: 294 $[M + H]^+$; Anal. Calcd. for $C_{17}H_{15}N_3O_2$: C, 69.61; H, 5.15; N, 14.33. Found: C, 69.36; H, 5.43; N, 14.70 (Alemany *et al*., 1966).

*N′-(2-hydroxy-5-methoxybenzylidene)-1H-indole-3-carbohydrazide (**3e**)* The derivative **3e** was obtained as a pale yellow solid by condensation of **B3** with 2-hydroxy-5-methoxybenzaldehyde in 97 % yield, mp 263–266 °C. IR (KBr) cm^{-1}: 3576 (ν OH), 3380–3260 (ν NH), 3200, 3058, 1663, 1640, 1614, 1577. ^1H NMR (500 MHz, DMSO-d_6): δ 11.79 (bs, 1H, Indole-NH), 11.69 (s, 1H, CONH), 10.88 (bs, 1H, OH), 8.50 (s, 1H, –N=C<u>H</u>–), 8.22 (bs, 1H, Indole H_2), 8.19 (d, 1H, $J = 7.80$ Hz, Indole H_4), 7.48 (d, 1H, $J = 7.80$ Hz, Indole H_7), 7.22–7.15 (m, 2H, Indole H_5, H_6), 7.12 (s, 1H, –N=CH–C_6<u>H_4</u>, H_6), 6.91–6.85 (m, 2H, –N=CH–C_6<u>H_4</u>, H_3, H_4), 3.74 (s, 3H, –OCH_3). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 160.77 (C=O), 159.10 (C2′ 2-hydroxy-5-methoxyphenyl), 151.17 (C5′ 2-hydroxy-5-methoxyphenyl), 144.97 (C=N), 136.08 (C3a), 128.77 (C2), 126.43 (C7a), 122.32 (C4), 121.00 (C6), 120.84 (C5), 119.26 (C1′ 2-hydroxy-5-methoxyphenyl), 117.38 (C4′ 2-hydroxy-5-methoxyphenyl), 117.12 (C3′ 2-hydroxy-5-methoxyphenyl), 112.46 (C6′ 2-hydroxy-5-methoxyphenyl), 112.02 (C3), 108.18 (C7), 55.43 (O-<u>C</u>H3). ESI-Mass *m/z*: 310 $[M + H]^+$, 332 $[M + Na]^+$; Anal. Calcd. for $C_{17}H_{15}N_3O_3$: C, 66.01; H, 4.89; N, 13.58. Found: C, 66.36; H, 5.03; N, 13.70.

*N′-(4-nitrobenzylidene)-1H-indole-3-carbohydrazide (**3f**)* The derivative **3f** was obtained as a yellow solid by condensation of **B3** with 4-nitrobenzaldehyde in 89 % yield, mp 289–291 °C. IR (KBr) cm^{-1}: 3387, 3250, 3126, 3044, 1638, 1584, 1552, 1520 and 1343 (NO_2); ^1H NMR (500 MHz, DMSO-d_6): δ 11.79 (bs, 2H, CONH, Indole-NH), 8.41 (bs, 1H, Indole H_2), 8.30–8.27 (m, 3H, –N=CH–C_6<u>H_5</u>, H_3, H_5, –N=C<u>H</u>–), 8.23 (d, 1H, $J = 7.7$ Hz, Indole H_4), 7.96 (d, 2H, $J = 8.6$ Hz, –N=CH–C_6<u>H_5</u>, H_2, H_6), 7.50 (d, 1H, $J = 7.7$ Hz, Indole H_7), 7.23–7.16 (m, 2H, Indole H_5, H_6). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 161.76 (C=O), 147.38 (C4′ 4-nitrophenyl), 141.57 (C=N), 141.18 (C1′ 4-nitrophenyl), 135.98 (C3a), 129.33 (C2), 127.50 (C7a), 126.67 (C2′ and C6′ 4-nitrophenyl), 124.00 (C3′ and C5′ 4-nitrophenyl), 122.35 (C4), 121.09 (C6), 120.90 (C5), 112.02 (C3), 108.03 (C7). ESI-Mass *m/z*: 309 $[M + H]^+$; Anal. Calcd. for $C_{16}H_{12}N_4O_3$: C, 62.33; H, 3.92; N, 18.17. Found: C, 62.54; H, 3.63; N, 18.52 (Alemany *et al*., 1966).

*N′-((1H-indol-3-yl) methylene)-1H-indole-3-carbohydrazide (**3g**)* The derivative **3g** was obtained as a white solid by condensation of **B3** with 1H-indole-3-carbaldehyde in 63.5 % yield, mp 279–281 °C. IR (KBr) cm^{-1}: 3387, 3361, 3210, 3110, 3068, 1632, 1607, 1577, 1541; ^1H NMR (500 MHz, DMSO-d_6): δ 12.03 (s, 1H, Indole-NH), 11.81 (s, 1H, –Indole-NH), 10.31 (s, 1H, CONH), 8.99 (s, 1H, –N=C<u>H</u>–), 8.60 (s, 1H, –Indole H_2), 7.68 (d, 1H, $J = 7.9$ Hz, –Indole H_4), 7.48 (d, 1H, $J = 8.1$ Hz, Indole H_4), 7.33 (s, 1H, Indole H_2), 7.24 (t, 2H, $J = 7.4$ Hz, –Indole H_7, Indole H_7), 7.04–7.09 (m, 2H –Indole H_5, Indole H_5), 6.79–6.73 (m, 2H, –Indole H_6, Indole H_6). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 157.34 (C=O), 150.05 (C=N), 149.89 (C3a), 146.57 (C3′a 1*H*-indole-3-yl), 136.85 (C2), 129.74 (C2′ 1*H*-indole-3-yl), 126.96 (C7a), 123.89 (C7′a 1*H*-indole-3-yl), 121.78 (C4), 119.98 (C4′ 1*H*-

indole-3-yl), 119.23 (C6), 118.82 (C6′ 1*H*-indole-3-yl), 118.23 (C5), 118.00 (C5′ 1*H*-indole-3-yl), 117.04 (C3), 113.44 (C3′ 1*H*-indole-3-yl), 112.37 (C7), 103.67 (C7′ 1*H*-indole-3-yl). ESI-Mass *m/z*: 303 $[M + H]^+$; Anal. Calcd. for $C_{18}H_{14}N_4O$: C, 71.51; H, 4.67; N, 18.53. Found: C, 71.16; H, 4.39; N, 18.89 (Alemany *et al*., 1966).

*N′-(thiophen-2-ylmethylene)-1H-indole-3-carbohydrazide (**3h**)* The derivative **3h** was obtained as a pale yellow solid by condensation of **B3** with thiophene-2-carbaldehyde in 93 % yield, mp 269–271 °C. IR (KBr) cm^{-1}: 3382–3200 (ν NH), 3156, 3123, 1644, 1612, 1600, 1575. ^{1}H NMR (500 MHz, DMSO-*d*$_6$): δ 11.79 (s, 1H, Indole-NH), 11.39 (s, 1H, CONH), 8.23–8.61 (m, 2H, Indole H_4, –N=C<u>H</u>–), 7.62 (d, 1H, $J = 4.0$ Hz, Thiophene H_5), 7.49 (d, 1H, $J = 7.8$ Hz, Indole H_7), 7.42 (s, 1H, $J = 4.0$ Hz, Thiophene H_3), 7.21 (t, 1H, $J = 7.6$ Hz, Indole H_5) 7.16 (t, 1H, $J = 7.6$ Hz, Indole H_6), 7.13 (t, 1H, $J = 4.0$ Hz, Thiophene H_4).^{13}C NMR (125 MHz, DMSO-*d*$_6$) *δ:* 161.03 (C=O), 140.56 (C2′ thiophen-2-yl), 139.70 (C3a), 135.91 (C2), 129.75 (C3′ thiophen-2-yl), 127.98 (C5′ thiophen-2-yl), 127.80 (C=N), 127.07 (C4′ thiophen-2-yl), 126.67 (C7a), 122.23 (C4), 121.13 (C6), 120.76 (C5), 111.92 (C3), 108.51 (C7). ESI-Mass *m/z*: 270 $[M + H]^+$, 292 $[M + Na]^+$; Anal. Calcd. for $C_{14}H_{11}N_3OS$: C, 62.44; H, 4.12; N, 15.60. Found: C, 62.06; H, 4.39; N, 15.92 (Alemany *et al*., 1966).

*N′-(5-chloro-2-nitrobenzylidene)-1H-indole-3-carbohydrazide (**3i**)* The derivative **3i** was obtained as a yellow solid by condensation of **B3** with 5-chloro-2-nitrobenzaldehyde in 90 % yield, mp 267–269 °C. IR (KBr) cm^{-1}: 3450–3335 (ν NH), 3189, 3086, 1646, 1607, 1581, 1563, 1530 and 1339 (ν NO_2). ^{1}H NMR (500 MHz, DMSO-*d*$_6$): δ 11.90 (s, 1H, Indole-NH), 11.86 (s, 1H, CONH), 8.74 (s, 1H, –N=C<u>H</u>–), 8.28 (d, 1H, $J = 2.35$ Hz, –N=CH–C_6<u>H_4</u> H_6), 8.21 (d, 1H, $J = 7.6$ Hz, –N=CH–C_6<u>H_4</u> H_3), 8.10–8.12 (m, 2H, Indole H_4, H_2), 7.69 (dd, 1H, $J = 8.7$, 2.95 Hz, –N=CH–C_6<u>H_4</u> H_4), 7.50 (d, 1H, $J = 8.0$ Hz, Indole H_7), 7.23–7.16 (m, 2H, Indole H_5, H_6). ^{13}C NMR (125 MHz, DMSO-*d*$_6$) *δ:* 161.25 (C=O), 146.26 (C=N), 138.28 (C2′ 5-chloro-2-nitrophenyl), 137.85 (C5′ 5-chloro-2-nitrophenyl), 136.00 (C3a), 131.35 (C2), 130.82 (C4′ 5-chloro-2-nitrophenyl), 129.55 (C1′ 5-chloro-2-nitrophenyl), 126.93 (C6′ 5-chloro-2-nitrophenyl), 126.78 (C7a), 126.60 (C3′ 5-chloro-2-nitrophenyl), 122.38 (C4), 121.03 (C6), 120.95 (C5), 112.03 (C3), 107.91 (C7). ESI-Mass *m/z*: 365 $[M + Na]^+$; Anal. Calcd. for $C_{16}H_{11}ClN_4O_3$: C, 56.07; H, 3.24; N, 16.35. Found: C, 55.82; H, 3.56; N, 16.80.

*N′-(2,3-dihydroxybenzylidene)-1H-indole-3-carbohydrazide (**3j**)* The derivative **3j** was obtained as a white-gray solid by condensation of **B3** with 2,3-dihydroxybenzaldehyde in 71 % yield, mp 229–231 °C. IR (KBr) cm^{-1}: 3737 and 3682 (ν OH), 3420–3225 (ν NH), 3146, 3077, 1675, 1623, 1589, 1573. ^{1}H NMR (500 MHz, DMSO-*d6*): δ 11.79 (s, 1H, Indole-NH), 11.70 (s, 1H, CONH), 11.41 (s, 1H, OH), 9.16 (s, 1H, OH), 8.47 (s, 1H, –N=C<u>H</u>–), 8.21–8.18 (m, 2H, Indole H_4, H_2), 7.49 (d, 1H, $J = 7.9$ Hz, Indole H_7), 7.21 (t, 1H, $J = 7.9$ Hz, Indole H_5), 7.17 (t, 1H, $J = 7.9$ Hz, Indole H_6), 6.95 (m, 1H, –N=CH–C_6<u>H_4</u>, H_6), 6.84 (d, 1H, $J = 7.8$ Hz, –N=CH–C_6<u>H_4</u>, H_4), 6.74 (t, 1H, $J = 7.8$ Hz, –N=CH–C_6<u>H_4</u>, H_5).^{13}C NMR (125 MHz, DMSO-*d6*) δ: 160.64 (C=O), 146.29 (C2′ 2,3-dihydroxyphenyl), 145.74 (C3′ 2,3-dihydroxyphenyl), 145.51 (C=N), 136.09 (C3a), 128.72 (C2), 126.33 (C7a), 122.33 (C4), 120.96 (C6), 120.85 (C5), 119.94 (C6′ 2,3-dihydroxyphenyl), 119.04 (C5′ 2,3-dihydroxyphenyl), 117.95 (C1′ 2,3-dihydroxyphenyl), 116.86 (C4′ 2,3-dihydroxyphenyl), 112.03 (C3), 108.13 (C7). ESI-Mass *m/z*: 296 $[M + H]^+$, 318 $[M + Na]^+$; Anal. Calcd. for $C_{16}H_{13}N_3O_3$: C, 65.08; H, 4.44; N, 14.23. Found: C, 65.32; H, 4.09; N, 14.56.

*N′-(2,5-dihydroxybenzylidene)-1H-indole-3-carbohydrazide (**3k**)* The derivative **3k** was obtained as a yellow-white solid by condensation of **B3** with 2,5-dihydroxybenzaldehyde in 81 % yield, mp 265–266 °C. IR (KBr) cm^{-1}: 3678 and 3576 (ν OH), 3390–3257 (ν NH), 3200, 3067, 1663, 1640, 1614, 1577. ^{1}H NMR (500 MHz, DMSO-*d6*): δ 11.78 (s, 1H, Indole-NH), 11.60 (s, 1H, CONH), 10.68 (s, 1H, OH_2), 8.97 (s, 1H, OH_5), 8.45 (s, 1H, –N=C<u>H</u>–), 8.21–8.19 (m, 2H, Indole H_4, H_2), 7.49 (d, 1H, $J = 7.8$ Hz, –N=CH–C_6<u>H_4</u>, H_4), 7.22–7.15 (m, 2H, Indole H_5, H_6), 6.95 (s, 1H, –N=CH–C_6<u>H_4</u>, H_6), 6.76 (d, 1H, $J = 8.7$ Hz, Indole H_7), 6.72 (dd, 1H, $J = 8.7$, 2.7 Hz, –N=CH–C_6<u>H_4</u>, H_3). ^{13}C NMR (125 MHz, DMSO-*d6*) δ: 160.60 (C=O), 150.09 (C2′ 2,5-dihydroxyphenyl), 149.79 (C5′ 2,5-dihydroxyphenyl), 145.22 (C=N), 136.06 (C3a), 128.61 (C2), 126.39 (C7a), 122.29 (C4), 120.99 (C6), 119.24 (C5), 118.97 (C3′ 2,5-dihydroxyphenyl), 118.19 (C1′ 2,5-dihydroxyphenyl), 116.92 (C4′ 2,5-dihydroxyphenyl), 114.00 (C6′ 2,5-dihydroxyphenyl), 112.00 (C3), 108.24 (C7). ESI-Mass *m/z*: 296 $[M + H]^+$, 318 $[M + Na]^+$; Anal. Calcd. for $C_{16}H_{13}N_3O_3$: C, 65.08; H, 4.44; N, 14.23. Found: C, 64.82; H, 4.73; N, 14.52.

*N′-(2,4-dihydroxybenzylidene)-1H-indole-3-carbohydrazide (**3l**)* The derivative **3l** was obtained as a pale yellow-white solid by condensation of **B3** with 2,4-dihydroxybenzaldehyde in 88 % yield, mp 293–295 °C. IR (KBr) cm^{-1}: 3658 and 3552 (ν OH), 3407, 3327, 3239, 3159, 1619, 1595, 1576, 1543. ^{1}H NMR (500 MHz, DMSO-*d6*): δ 11.77 (s, 1H, Indole-NH), 11.71(s, 1H, CONH), 11.56 (s, 1H, OH_2), 9.93 (s, 1H, OH_4), 8.44 (s, 1H, –N=C<u>H</u>–), 8.23–8.21 (m, 2H, Indole H_4, H_2), 7.50 (d, 1H, $J = 7.8$ Hz, –N=CH–C_6<u>H_4</u>, H_6), 7.30 (d, 1H, $J = 3.0$ Hz, –N=CH–C_6<u>H_4</u>, H_3), 7.22–7.16 (m, 2H, Indole H_5,H_6), 6.42–6.39 (m,

2H, –N=CH–$C_6\underline{H}_4$, H_5, Indole H_7). ^{13}C NMR (125 MHz, DMSO-*d6*) δ: 160.57 (C=O), 160.21 (C2′ 2,4-dihydroxyphenyl), 159.23 (C4′ 2,4-dihydroxyphenyl), 146.72 (C=N), 136.11 (C3a), 131.08 (C6′ 2,4-dihydroxyphenyl), 128.47 (C2), 126.34 (C7a), 122.28 (C4), 120.98 (C6), 120.79 (C5), 112.01 (C3), 110.85 (C1′ 2,4-dihydroxyphenyl), 108.33 (C7), 107.52 (C5′ 2,4-dihydroxyphenyl), 102.69 (C3′ 2,4-dihydroxyphenyl). ESI-Mass *m/z*: 296 $[M + H]^+$, 318 $[M + Na]^+$; Anal. Calcd. for $C_{16}H_{13}N_3O_3$: C, 65.08; H, 4.44; N, 14.23. Found: C, 65.41; H, 4.02; N, 14.46.

*N′-(2,3-dimethoxybenzylidene)-1H-indole-3-carbohydrazide (**3m**)* The derivative **3m** was obtained as an off-white solid by condensation of **B3** with 2,3-dimethoxybenzaldehyde in 85 % yield, mp 224–226 °C. IR (KBr) cm^{-1}: 3417–3272, 3197, 3094, 1652, 1619, 1615, 1544. ^{1}H NMR (500 MHz, DMSO-*d6*): δ 11.84 (s, 1H, Indole-NH), 11.56 (s, 1H, CONH), 8.62 (bs, 1H, –N=C$\underline{H}$–), 8.26 (bs, 2H, Indole H_4, H_2), 7.51–7.49 (m, 2H, –N=CH–$C_6\underline{H}_4$, H_6, Indole H_7), 7.22–7.16 (m, 2H, –N=CH–$C_6\underline{H}_4$, H_5, Indole H_5), 7.12 (t, 1H, $J = 8.0$ Hz, Indole H_6), 7.07 (d, 1H, $J = 7.9$ Hz, –N=CH–$C_6\underline{H}_4$, H_4) 3.83 (s, 3H, OC$\underline{H}$3), 3.75 (s, 3H, OC$\underline{H}$3). ^{13}C NMR (125 MHz, DMSO-*d6*): δ 161.04 (C=O), 152.63 (C3′ 2,3-dimethoxyphenyl), 147.67 (C2′ 2,3-dimethoxyphenyl), 140.09 (C=N), 135.96 (C3a), 131.51 (C2), 128.20 (C7a), 126.60 (C6′ 2,3-dimethoxyphenyl), 124.28 (C1′ 2,3-dimethoxyphenyl), 122.21 (C4), 121.16 (C6), 120.74 (C5), 116.89 (C4′ 2,3-dimethoxyphenyl), 113.63 (C5′ 2,3-dimethoxyphenyl), 110.92 (C3), 108.48 (C7), 61.09(O-$\underline{C}$H3), 55.68 (O-$\underline{C}$H3). ESI-Mass *m/z*: 324 $[M + H]^+$, 346 $[M + Na]^+$; Anal. Calcd. for $C_{18}H_{17}N_3O_3$: C, 66.86; H, 5.30; N, 13.00. Found: C, 66.52; H, 5.58; N, 12.79.

*N′-(3,4,5-trimethoxybenzylidene)-1H-indole-3-carbohydrazide (**3n**)* The derivative **3n** was obtained as a white solid by condensation of **B3** with 3, 4, 5-trimethoxybenzaldehyde in 96 % yield, mp 245–246 °C. IR (KBr) cm^{-1}: 3200–3111, 3048, 3005, 1686, 1639, 1617, 1571. ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.78 (s, 1H, CONH), 11.42 (s, 1H, Indole-NH), 8.29–8.21 (m,3H, Indole H_2, –N=C$\underline{H}$–, Indole H_4), 7.49 (d, 1H, $J = 8.0$ Hz, Indole H_7), 7.22–7.15 (m, 2H, Indole H_5, H_6), 7.02 (s, 2H, –N=CH–$C_6\underline{H}_4$, H_2, H_6), 3.89 (s, 6H, OC$\underline{H}$3), 3.76 (s, 3H, OC$\underline{H}$3). ^{13}C NMR (125 MHz, DMSO-d_6): δ 161.04 (C=O), 153.17 (C3′ and C5′ 3,4,5-trimethoxyphenyl), 144.78 (C=N), 143.85 (C4′ 3,4,5-trimethoxyphenyl), 135.91 (C3a), 130.33 (C2), 128.49 (C7a), 126.62 (C1′ 3,4,5-trimethoxyphenyl), 122.18 (C4), 121.13 (C6), 120.71 (C5), 111.92 (C3), 108.42 (C7), 103.89 (C2′ and C6′ 3,4,5-trimethoxyphenyl), 60.08 (C4′ O-CH3), 55.85 (C3′ and C5′ O-CH3). ESI-Mass *m/z*: 354 $[M + H]^+$, 393 $[M + K]^+$; Anal. Calcd. for $C_{19}H_{19}N_3O_4$: C, 64.58; H, 5.42; N, 11.89. Found: C, 64.12; H, 5.73 N, 12.05.

*N′-(3-nitrobenzylidene)-1H-indole-3-carbohydrazide (**3o**)* The derivative **3o** was obtained as a yellow solid by condensation of **B3** with 3-nitrobenzaldehyde in 98 % yield, mp 278–281 °C. IR (KBr) cm^{-1}: 3531, 3461, 3285, 3117, 1629, 1591, 1577, 1541 and 1364 (NO_2). ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.84 (s, 1H, Indole-NH), 11.69 (s, 1H, CONH), 8.51 (s, 1H, –N=CH–$C_6\underline{H}_5$, H_2), 8.43 (bs, 1H, –N=C$\underline{H}$–),8.31 (bs, 1H, Indole H_2), 8.25 (d, 1H, $J = 7.6$ Hz, Indole H_4), 8.19 (d, 1H, $J = 7.7$ Hz, –N=CH–$C_6\underline{H}_5$ H_6) 8.10 (d, 1H, $J = 7.5$ Hz, –N=CH–$C_6\underline{H}_5$ H_4), 7.68 (t, 1H, $J = 7.7$ Hz, –N=CH–$C_6\underline{H}_5$, H_5), 7.49 (d, 1H, $J = 7.6$ Hz, Indole H_7), 7.22–7.16 (m, 2H, Indole H_5, H_6). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 161.43 (C=O), 148.17 (C3′ 3-nitrophenyl), 141.93 (C=N), 136.65 (C3a), 136.00 (C1′ 3-nitrophenyl), 132.89 (C6′ 3-nitrophenyl), 130.25 (C2), 129.16 (C7a), 126.66 (C5′ 3-nitrophenyl), 123.56 (C4′ 3-nitrophenyl), 122.20 (C4), 121.10 (C6), 120.86 (C2′ 3-nitrophenyl), 120.50 (C5), 111.99 (C3), 108.21 (C7). ESI-Mass *m/z*: 309 $[M + H]^+$, 331 $[M + Na]^+$; Anal. Calcd. for $C_{16}H_{12}N_4O_3$: C, 62.33; H, 3.92; N, 18.17. Found: C, 62.64; H, 3.83; N, 17.92.

*N′-((5-nitrothiophen-2-yl)methylene)-1H-indole-3-carbohydrazide (**3p**)* The derivative **3p** was obtained as a yellow-orange solid by condensation of **B3** with 5-nitrothiophene-2-carbaldehyde in 93 % yield, mp 285–286 °C. IR (KBr) cm^{-1}: 3425, 3232, 3119, 3067, 1653, 1592, 1573, 1536 and 1334 (NO_2). ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.88 (s, 1H, Indole-NH), 11.81 (s, 1H, CONH), 8.56 (bs, 1H,–N=C$\underline{H}$–), 8.26 (s, 1H, Indole H_2), 8.20 (d, 1H, $J = 7.7$ Hz, Indole H_4), 8.14 (d, 1H, $J = 4.4$ Hz, Thiophene H_4), 7.54 (d, 1H, $J = 4.4$, Thiophene H_3), 7.49 (d, 1H, $J = 7.7$ Hz, Indole H_7), 7.23–7.16 (m, 2H, Indole H_5, H_6). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 166.95 (C=O), 155.32 (C5′ 5-nitrothiophen-2-yl), 152.88 (C2′ 5-nitrothiophen-2-yl), 135.83 (C3a), 133.74 (C3′ 5-nitrothiophen-2-yl), 132.98 (C4′ 5-nitrothiophen-2-yl), 131.21 (C2), 129.85 (C7a), 128.72 (C=N), 122.67 (C4), 121.29 (C6), 120.61 (C5), 112.28 (C3), 108.07 (C7). ESI-Mass *m/z*: 315 $[M + H]^+$, 337 $[M + Na]^+$; Anal. Calcd. for $C_{14}H_{11}N_3OS$: C, 53.50; H, 3.21; N, 17.83. Found: C, 53.16; H, 2.99; N, 17.98.

*N′-((5-nitrofuran-2-yl)methylene)-1H-indole-3-carbohydrazide (**3q**)* The derivative **3q** was obtained as a yellow solid by condensation of **B3** with 5-nitrofuran-2-carbaldehyde in 98 % yield, mp 285–286 °C. IR (KBr) cm^{-1}: 3420–3372, 3188, 3120, 1633, 1586, 1566, 1523 and 1355 (NO_2). ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.88 (s, 1H, Indole-NH), 11.81 (s, 1H, CONH), 8.26 (s, 1H,–N=C$\underline{H}$–), 8.21 (d, 1H, $J = 8.0$ Hz, Indole H_4), 7.81 (s, 1H, Indole H_2), 7.49 (d, 1H, $J = 6.8$ Hz, Furan H_4), 7.19–7.23 (m, 3H, Indole H_5,

H_6, H_7), 7.16 (d, 1H, $J = 7.2$ Hz, Furan H_3). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 161.65 (C=O), 152.46 (C2′ 5-nitrofuran-2-yl), 151.63 (C5′ 5-nitrofuran-2-yl), 135.94 (C3a), 131.51 (C=N), 130.17 (C2), 126.66 (C7a), 122.43 (C4), 121.02 (C6), 117.18 (C5), 114.88 (C3′ 5-nitrofuran-2-yl), 114.13 (C4′ 5-nitrofuran-2-yl), 112.04 (C3), 107.84 (C7). ESI-Mass *m/z*: 299 $[M + H]^+$, 321 $[M + Na]^+$; Anal. Calcd. for $C_{14}H_{11}N_3OS$: C, 56.38; H, 3.38; N, 18.79. Found: C, 56.02; H, 3.79; N, 18.52.

N′-(3-methoxybenzylidene)-1H-indole-3-carbohydrazide ***(3r)*** The derivative **3r** was obtained as a pale pink-white solid by condensation of **B3** with 3-methoxybenzaldehyde in 72 % yield, mp 239–241 °C. IR (KBr) cm^{-1}: 3252, 3156, 3063, 3009, 1625, 1618, 1603, 1573. ^1H NMR (500 MHz, DMSO-d_6): δ 11.75 (s, 1H, Indole-NH), 11.36 (s, 1H, CONH), 8.28–8.19 (m, 3H, Indole H_2, H_4, –N=CH–), 7.54 (s, 1H, –N=CH–C_6H_5, H_2), 7.51–7.48 (m, 2H, –N=CH–C_6H_5, H_6, Indole H_7), 7.35 (t, 1H, $J = 7.6$ Hz, –N=CH–C_6H_5, H_5), 7.23 (t, 1H, $J = 7.7$ Hz, Indole H_5), 7.19 (t, 1H, $J = 7.7$ Hz, Indole H_6), 7.15 (d, 1H, $J = 7.6$ Hz, –N=CH–C_6H_5, H_4), 2.37 (s, 3H, $–CH_3$). ^{13}C NMR (125 MHz, DMSO-d_6): δ 162.14 (C=O), 161.09 (C3′ 3-methoxyphenyl), 148.65 (C=N), 140.13 (C3a), 138.33 (C1′ 3-methoxyphenyl), 130.24 (C2), 129.75 (C5′ 3-methoxyphenyl), 127.43 (C7a), 122.55 (C4), 122.14 (C6′ 3-methoxyphenyl), 121.89 (C6), 119.57 (C5), 117.02 (C4′ 3-methoxyphenyl), 112.39 (C2′ 3-methoxyphenyl), 110.09 (C3), 108.99 (C7), 54.29 (O-CH3). ESI-Mass *m/z*: 294 $[M + H]^+$, 316 $[M + Na]^+$; Anal. Calcd. for $C_{17}H_{15}N_3O_2$: C, 69.61; H, 5.15; N, 14.33. Found: C, 69.49; H, 4.87; N, 13.98.

N′-benzylidene-1H-indole-2-carbohydrazide ***(2a)*** The derivative **2a** was obtained as an off-white solid by condensation of **B2** with benzaldehyde in 95 % yield, mp 197–199 °C. IR (KBr) cm^{-1}: 3404, 3264, 3125, 3064, 1656, 1623, 1610, 1593, 1575; ^1H NMR (500 MHz, DMSO-d_6): δ 11.90 (s, 1H, CONH), 11.80 (s, 1H, Indole-NH), 8.47 (s, 1H, –N=CH–), 7.77 (d, 2H, $J = 7.1$ Hz, –N=CH–C_6H_5, H_2, H_6), 7.69 (d, 1H, $J = 7.2$ Hz, Indole H_7), 7.49–7.44 (m, 4H, Indole H_4, –N=CH–C_6H_5, H_3,H_4,H_5), 7.33 (s, 1H, Indole H_3), 7.23 (t, 1H, $J = 7.2$ Hz, Indole H_5), 7.08 (t, 1H, $J = 7.2$ Hz, Indole H_6). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 161.11 (C=O), 157.63 (C=N), 147.08 (C7a), 144.63 (C2), 136.81 (C1′ benzyl), 134.30 (C3a), 129.98 (C4′ benzyl), 128.83 (C2′ and C6′ benzyl), 127.03 (C3′ and C5′ benzyl), 123.84 (C6), 121.75 (C4), 119.94 (C5), 112.36 (C3), 103.58 (C7). ESI-Mass *m/z*: 264 $[M + H]^+$, 286 $[M + Na]^+$; Anal. Calcd. for $C_{16}H_{13}N_3O$: C, 72.99; H, 4.98; N, 15.96. Found: C, 72.52; H, 5.08; N, 15.72 (Alemany *et al*., 1966).

N′-(2,4-dichlorobenzylidene)-1H-indole-2-carbohydrazide ***(2b)*** The derivative **2b** was obtained as a pale yellow-white solid by condensation of **B2** with 2,4-dichlorobenzaldehyde in 98 % yield, as a 80:20 mixture of *E* and *Z* isomers, mp 228–230 °C. IR (KBr) cm^{-1}: 3472, 3316, 3126, 3103, 1659, 1612, 1594,1569.1540; ^1H NMR (500 MHz, DMSO-d_6): δ 12.18 (s, 1H, CONH), 11.86 (s, 1H, Indole-NH), 8.80 (s, 1H, –N=CH–), 8.05 (d, 1H, $J = 8.5$ Hz, –N=CH–C_6H_5, H_6 (*E*)), 7.86 (d, 1H, $J = 8.5$ Hz, –N=CH–C_6H_5, H_6 (*Z*)), 7.81 (d, 1H, $J = 2.1$ Hz, –N=CH–C_6H_5, H_3 (*Z*)), 7.71 (d, 1H, $J = 2.1$ Hz, –N=CH–C_6H_5, H_3 (*E*)), 7.69 (d, 1H, $J = 8.1$ Hz, Indole H_7), 7.61 (dd, 1H, $J = 7.4$, 2.1 Hz, –N=CH–C_6H_5, H_5 (*Z*)), 7.53 (d, 1H, $J = 7.4$ Hz, –N=CH–C_6H_5, H_5 (*E*)), 7.48 (d, 1H, $J = 7.8$ Hz, Indole H_4), 7.36 (s, 1H, Indole H_3), 7.24 (t, 1H, $J = 7.8$ Hz, Indole H_6), 7.08 (t, 1H, $J = 7.8$ Hz, Indole H_5). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 157.68 (C=O), 141.74 (C7a), 139.70 (C=N), 136.92 (C2), 134.94 (C1′ 2,4-dichlorophenyl), 133.74 (C3a), 131.02 (C2′ 2,4-dichlorophenyl (*Z*)), 130.70 (C2′ 2,4-dichlorophenyl (*E*)), 130.23 (C3′ 2,4-dichlorophenyl (*Z*)), 129.68 (C6′ 2,4-dichlorophenyl (*Z*)), 129.34 (C3′ 2,4-dichlorophenyl (*E*)), 128.23 (C6′ 2,4-dichlorophenyl (*E*)), 128.03 (C4′ 2,4-dichlorophenyl), 126.93 (C5′ 2,4-dichlorophenyl), 124.03 (C6), 121.85 (C4), 120.00 (C5), 112.40 (C3), 103.96 (C7). ESI-Mass *m/z*: 332 $[M + H]^+$, 354 $[M + Na]^+$; Anal. Calcd. for $C_{16}H_{11}Cl_2N_3O$: C, 57.85; H, 3.34; N, 12.65. Found: C, 58.03; H, 3.18; N, 12.92.

N′-(2,6-dichlorobenzylidene)-1H-indole-2-carbohydrazide ***(2c)*** The derivative **2c** was obtained as a white solid by condensation of **B2** with 2,6-dichlorobenzaldehyde in 98 % yield, mp 224–227 °C. IR (KBr) cm^{-1}: 3426, 3283, 3103, 3023, 1667, 1624, 1610, 1574,1547; ^1H NMR (500 MHz, DMSO-d_6): δ 12.19 (s, 1H, CONH), 11.84 (s, 1H, Indole-NH), 8.66 (s, 1H, –N=CH–), 7.69 (d, 1H, $J = 8.1$ Hz, Indole H_7), 7.58 (d, 2H, $J = 8.5$ Hz, –N=CH–C_6H_5, H_3, H_5), 7.49 (d, 1H, $J = 8.1$ Hz, Indole H_4),7.45 (t, 1H, $J = 8.5$ Hz, –N=CH–C_6H_5, H_4), 7.38 (bs, 1H, Indole H_3), 7.24 (t, 1H, $J = 8.1$ Hz, Indole H_6), 7.08 (d, 1H, $J = 8.1$, Indole H_5). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 157.76 (C=O), 142.35 (C7a), 136.92 (C=N), 134.53 (C2), 133.90 (C2′ and C6′ 2,6-dichlorophenyl), 131.11 (C3a), 130.65 (C1′ 2,6-dichlorophenyl), 129.92 (C4′ 2,6-dichlorophenyl), 129.05 (C3′ and C5′ 2,6-dichlorophenyl), 124.04 (C6), 121.89 (C4), 119.98 (C5), 112.40 (C3), 104.03 (C7). ESI-Mass *m/z*: 332 $[M + H]^+$, 354 $[M + Na]^+$; Anal. Calcd. for $C_{16}H_{11}Cl_2N_3O$: C, 57.85; H, 3.34; N, 12.65. Found: C, 57.63; H, 3.48; N, 13.02.

N′-(4-methoxybenzylidene)-1H-indole-2-carbohydrazide ***(2d)*** The derivative **2d** was obtained as a white solid by condensation of **B2** with 4-methoxybenzaldehyde in 94 % yield, mp 213–215 °C. IR (KBr) cm^{-1}: 3442–3209, 3109,

3053, 1669, 1635, 1596, 1577, 1541; ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.77 (s, 1H, CONH), 11.62 (s, 1H, Indole-NH), 8.40 (s, 1H, –N=CH–), 7.71 (d, 2H, J = 8.4 Hz, –N=CH–C_6H_5, H_2, H_6), 7.68 (d, 1H, J = 7.9 Hz, Indole H_7), 7.47 (d, 1H, J = 7.9 Hz, Indole H_4), 7.30 (s, 1H, Indole H_3), 7.22 (t, 1H, J = 7.9 Hz, Indole H_6), 7.09–7.04 (m, 3H, –N=CH–C_6H_5, H_3, H_5, Indole H_5), 3.83 (s, 3H, –CH_3). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 160.77 (C4′ 4-methoxyphenyl), 157.50 (C=O), 147.04 (C=N), 136.77 (C7a), 130.18 (C2), 128.65 (C3a), 126.98 (C2′ and C6′ 4-methoxyphenyl), 126.86 (C1′ 4-methoxyphenyl), 123.73 (C6), 121.68 (C4), 119.91 (C5), 114.32 (C3′ and C5′ 4-methoxyphenyl), 112.35 (C3), 103.35 (C7), 55.26 (O-CH3). ESI-Mass *m/z*: 294 $[M + H]^+$, 316 $[M + Na]^+$; Anal. Calcd. for $C_{17}H_{15}N_3O_2$: C, 69.61; H, 5.15; N, 14.33. Found: C, 69.36; H, 5.43; N, 14.70 (Alemany *et al*., 1966).

*N′-((5-nitrothiophen-2-yl)methylene)-1H-indole-2-carbohydrazide (**2e**)* The derivative **2e** was obtained as a primrose yellow solid by condensation of **B2** with 5-nitrothiophene-2-carbaldehyde in 88 % yield, mp 275–276 °C. IR (KBr) cm^{-1}: 3420, 3304, 3237, 3214, 1628, 1584, 1559, 1548, 1524 and 1353 (NO_2); ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.28 (s, 1H, CONH), 11.86 (s, 1H, Indole-NH), 8.69 (s, 1H, –N=CH–), 8.16 (d, 1H, J = 4.3 Hz, Thiophene H_4), 7.70 (d, 1H, J = 7.8 Hz, Indole H_7), 7.61 (d, 1H, J = 4.3 Hz, Thiophene H_3), 7.48 (d, 1H, J = 7.8 Hz, Indole H_4), 7.36 (s, 1H, Indole H_3), 7.25 (t, 1H, J = 7.8 Hz, Indole H_5), 7.09 (t, 1H, J = 7.8 Hz, Indole H_6). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 157.74 (C=O), 150.67 (C5′ 5-nitrothiophen-2-yl), 146.73 (C2′ 5-nitrothiophen-2-yl), 140.35 (C7a), 137.00 (C2), 130.49 (C3a), 129.44 (C4′ 5-nitrothiophen-2-yl), 126.89 (C3′ 5-nitrothiophen-2-yl), 124.23 (C=N), 122.19 (C6), 121.94 (C4), 120.09 (C5), 112.42 (C3), 104.37 (C7). ESI-Mass *m/z*: 315 $[M + H]^+$, 337 $[M + Na]^+$; Anal. Calcd. for $C_{14}H_{10}N_4O_3S$: C, 53.50; H, 3.21; N, 17.83. Found: C, 53.87; H, 3.60; N, 17.72.

*N′-(2-hydroxy-5-methoxybenzylidene)-1H-indole-2-carbohydrazide (**2f**)* The derivative **2f** was obtained as a pale yellow solid by condensation of **B2** with 2-hydroxy-5-methoxybenzaldehyde in 93 % yield, mp 276–278 °C. IR (KBr) cm^{-1}: 3594 (ν OH), 3431–3300 (ν NH), 3097, 3041, 1641, 1598, 1571, 1540,1512; ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.10 (s, 1H, CONH), 11.80 (s, 1H, Indole-NH), 10.59 (s, 1H, OH), 8.63 (s, 1H, –N=CH–), 7.68 (d, 1H, J = 8.0 Indole H_7), 7.47 (d, 1H, J = 8.0 Hz, Indole H_4), 7.33 (s, 1H, Indole H_3), 7.22 (t, 1H, J = 8.0 Hz, Indole H_6), 7.16 (s, 1H, –N=CH–C_6H_4, H_6), 7.07 (t, 1H, J = 8.0 Hz, Indole H_5), 6.91 (dd, 1H, J = 8.8, 3.0, –N=CH–C_6H_4, H_4), 6.86 (d, 1H, J = 8.8, –N=CH–C_6H_4, H_3), 3.74 (s, 3H, –CH_3). ^{13}C NMR (125 MHz, DMSO-d_6): δ 157.43 (C=O), 152.15 (C2′ 2-hydroxy-5-methoxyphenyl), 151.33 (C5′ 2-hydroxy-5-methoxyphenyl), 146.60 (C=N), 136.88 (C7a), 129.65 (C2), 126.95 (C3a), 123.95 (C6), 121.80 (C4), 120.01 (C5), 119.14 (C1′ 2-hydroxy-5-methoxyphenyl), 118.11 (C4′ 2-hydroxy-5-methoxyphenyl), 117.23 (C3′ 2-hydroxy-5-methoxyphenyl), 112, 39 (C3), 111.94 (C6′ 2-hydroxy-5-methoxyphenyl), 103.84 (C7), 55.47 (O-CH3). ESI-Mass *m/z*: 310 $[M + H]^+$, 332 $[M + Na]^+$; Anal. Calcd. for $C_{17}H_{15}N_3O_3$: C, 66.01; H, 4.89; N, 13.58. Found: C, 66.36; H, 5.03; N, 13.70.

*N′-(2,4-dihydroxybenzylidene)-1H-indole-2-carbohydrazide (**2g**)* The derivative **2g** was obtained as a pale yellow solid by condensation of **B2** with 2,4-dihydroxybenzaldehyde in 95 % yield, mp 292–293 °C. IR (KBr) cm^{-1}: 3571, 3499 (ν OH), 3410–3158 (ν NH), 3133, 3061, 1658, 1625, 1601, 1576, 1543. ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.96 (s, 1H, CONH), 11.79 (s, 1H, Indole-NH), 11.38 (s, 1H, OH_2), 9.96 (s, 1H, OH_4), 8.52 (s, 1H, –N=CH–), 7.68 (d, 1H, J = 8.0 Hz, Indole H_7), 7.47 (d, 1H, J = 8.0 Hz, Indole H_4), 7.35 (d, 1H, J = 8.4 Hz, –N=CH–C_6H_4, H_6), 7.29 (s, 1H, Indole H_3), 7.23 (t, 1H, J = 8.0 Hz, Indole H_6), 7.07 (t, 1H, J = 8.0 Hz, Indole H_5), 6.38 (dd, 1H, J = 8.5, 2.2 Hz, –N=CH–C_6H_4, H_5), 6.32 (s, 1H, –N=CH–C_6H_4, H_3).

^{13}C NMR (125 MHz, DMSO-d_6) δ: 160.63 (C2′ 2,4-dihydroxyphenyl), 159.29 (C4′ 2,4-dihydroxyphenyl), 157.09 (C=O), 148.21 (C=N), 136.79 (C7a), 131.02 (C2′ 2,4-dihydroxyphenyl), 129.79(C2), 126.96 (C3a), 123.78 (C6), 121.72 (C4), 119.95 (C5), 112.35 (C3), 110.65 (C1′ 2,4-dihydroxyphenyl), 107.69 (C5′ 2,4-dihydroxyphenyl), 103.42 (C7), 102.69 (C3′ 2,4-dihydroxyphenyl). ESI-Mass *m/z*: 296 $[M + H]^+$, 318 $[M + Na]^+$; Anal. Calcd. for $C_{16}H_{13}N_3O_3$: C, 65.08; H, 4.44; N, 14.23. Found: C, 64.82; H, 4.75; N, 14.83.

*N′-(2,5-dihydroxybenzylidene)-1H-indole-2-carbohydrazide (**2h**)* The derivative **2h** was obtained as a green-gray solid by condensation of **B2** with 2,5-dihydroxybenzaldehyde in 95 % yield, mp 304–305 °C. IR (KBr) cm^{-1}: 3572 (ν OH), 3413, 3337, 3127, 3100, 1699, 1651, 1614, 1575; ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.01 (s, 1H, CONH), 11.81 (s, 1H, Indole-NH), 10.27 (s, 1H, OH), 8.98 (s, 1H, OH), 8.58 (s, 1H, –N=CH–), 7.67 (d, 1H, J = 8.0 Hz, Indole H_7), 7.46 (d, 1H, J = 8.0 Hz, Indole H_4), 7.31 (s, 1H, Indole H_3), 7.22 (t, 1H, J = 8.0 Hz, Indole H_6), 7.07 (t, 1H, J = 8.0 Hz, Indole H_5), 7.03 (d, 1H, J = 2.6 Hz, –N=CH–C_6H_4, H_6), 6.76 (d, 1H, J = 8.7 Hz, –N=CH–C_6H_4, H_3), 6.71 (dd, 1H, J = 8.7, 2.6 Hz, –N=CH–C_6H_4, H_4). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 161.77 (C=O), 149.91 (C2′ 2,5-dihydroxyphenyl), 146.93 (C5′ 2,5-dihydroxyphenyl), 144.28 (C=N), 137.91 (C7a), 130.51 (C2), 127.13 (C3a), 122.54 (C6), 120.82 (C3′ 2,5-dihydroxyphenyl), 120.41 (C4), 119.09 (C5), 118.65 (C1′ 2,5-dihydroxyphenyl), 117.93 (C4′ 2,5-dihydroxyphenyl), 117.05 (C6′ 2,5-

dihydroxyphenyl), 113.39 (C3), 104.51 (C7). ESI-Mass *m/z*: 296 $[M + H]^+$, 318 $[M + Na]^+$, 334 $[M + K]^+$; Anal. Calcd. for $C_{16}H_{13}N_3O_3$: C, 65.08; H, 4.44; N, 14.23. Found: C, 65.53; H, 4.12; N, 14.62.

*N′-(2,3-dihydroxybenzylidene)-1H-indole-2-carbohydrazide (**2i**)* The derivative **2i** was obtained as a gray solid by condensation of **B2** with 2,3-dihydroxybenzaldehyde in 89 % yield, mp 238–240 °C. IR (KBr) cm^{-1}: 3799 and 3671 (ν OH), 3406, 3267, 3044, 1644, 1605, 1577, 1542. ^1H NMR (500 MHz, DMSO-d_6): δ 11.05 (s, 1H, OH_2), 10.51 (s, 1H, Indole-NH), 10.21 (s, 1H, CONH), 9.29 (s, 1H, OH_3), 8.60 (s, 1H, –N=CH–), 7.71 (d, 1H, $J = 7.4$ Hz, Indole H_7), 7.51 (d, 1H, $J = 7.4$ Hz, Indole H_4), 7.33 (s, 1H, Indole H_3), 7.22 (d, 1H, $J = 7.4$ Hz, Indole H_6), 7.04 (d, 1H, $J = 7.4$ Hz, Indole H_5), 6.91–6.65 (m, 3H, –N=CH–C_6H_4, H_4, H_5, H_6). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 157.37 (C=O), 148.01(C2′ 2,3-dihydroxyphenyl), 145.93 (C3′ 2,3-dihydroxyphenyl), 145.58 (C=N), 136.90 (C7a), 129.57 (C2), 126.93 (C3a), 123.94 (C6), 121.82 (C6′ 2,3-dihydroxyphenyl), 120.01 (C4), 119.79 (C5′ 2,3-dihydroxyphenyl), 119.15 (C5), 118.93 (C1′ 2,3-dihydroxyphenyl), 117.25 (C4′ 2,3-dihydroxyphenyl), 112.39 (C3), 103.80 (C7). ESI-Mass *m/z*: 296 $[M + H]^+$, 318 $[M + Na]^+$; Anal. Calcd. for $C_{16}H_{13}N_3O_3$: C, 65.08; H, 4.44; N, 14.23. Found: C, 65.42; H, 4.24; N, 13.92.

*N′-(5-chloro-2-nitrobenzylidene)-1H-indole-2-carbohydrazide (**2j**)* The derivative **2j** was obtained as a yellow solid by condensation of **B2** with 5-chloro-2-nitrobenzaldehyde in 92 % yield, as a 84:16 mixture of *E* and *Z* isomers, mp 278–280 °C. IR (KBr) cm^{-1}: 3424, 3265, 3078, 1655, 1613, 1544, 1540 and 1378 (NO_2); ^1H NMR (500 MHz, DMSO-d_6): δ 12.36 (s, 1H, Indole-NH), 11.87 (s, 1H, CONH), 10.21 (s, 1H, –N=CH– (*Z*)), 8.87 (s, 1H, –N=CH– (*E*)), 8.18 (d, 1H, $J = 8.7$ Hz, –N=CH–C_6H_4 H_3 (*Z*)), 8.15 (d, 1H, $J = 8.7$ Hz, –N=CH–C_6H_4 H_3 (*E*)), 8.11 (d, 1H, $J = 1.9$ Hz, –N=CH–C_6H_4 H_6 (*E*)), 8.05 (d, 1H, $J = 1.9$ Hz, –N=CH–C_6H_4 H_6 (*Z*)), 7.95 (dd, 1H, $J = 8.7$, 1.9 Hz, –N=CH–C_6H_4 H_4 (*Z*)), 7.76 (dd, 1H, $J = 8.7$, 1.9 Hz, –N=CH–C_6H_4 H_4 (*E*)), 7.69 (d, 1H, $J = 7.5$ Hz, Indole H_7), 7.48 (d, 1H, $J = 7.5$ Hz, Indole H_4), 7.39 (s, 1H, Indole H_3), 7.25 (t, 1H, $J = 7.5$ Hz, Indole H_6), 7.08 (t, 1H, $J = 7.5$ Hz, Indole H_5).^{13}C NMR (125 MHz, DMSO-d_6): δ 158.67 (C=O), 146.46 (C2′ 5-chloro-2-nitrophenyl (*E*)), 140.93 (C=N), 138.98 (C2′ 5-chloro-2-nitrophenyl (*Z*)), 138.40 (C7a), 136.98 (C2), 133.53 (C5′ 5-chloro-2-nitrophenyl (*E*)), 132.42(C5′ 5-chloro-2-nitrophenyl (*Z*)), 132.27 (C4′ 5-chloro-2-nitrophenyl (*Z*)), 131.39(C6′ 5-chloro-2-nitrophenyl (*Z*)), 130.97 (C4′ 5-chloro-2-nitrophenyl (*E*)), 130.06 (C6′ 5-chloro-2-nitrophenyl (*E*)), 129.42(C1′ 5-chloro-2-nitrophenyl (*Z*)), 129.18 (C1′ 5-chloro-2-nitrophenyl (*E*)), 126.99 (C3a), 126.90 (C3′ 5-chloro-2-nitrophenyl (*Z*)), 126.45 (C3′ 5-chloro-2-nitrophenyl (*E*)), 124.17 (C6), 121.92 (C4), 120.06 (C5), 112.42 (C3), 104.36 (C7). ESI-Mass *m/z*: 343 $[M + H]^+$, 365 $[M + Na]^+$; Anal. Calcd. for $C_{16}H_{11}ClN_4O_3$: C, 56.07; H, 3.24; N, 16.35. Found: C, 56.33; H, 3.02; N, 16.79.

*N′-((5-nitrofuran-2-yl)methylene)-1H-indole-2-carbohydrazide (**2k**)* The derivative **2k** was obtained as a yellow solid by condensation of **B2** with 5-nitrofuran-2-carbaldehyde in 87 % yield, as a 86:14 mixture of *E* and *Z* isomers, mp 273–274 °C. IR (KBr) cm^{-1}: 3482, 3363, 3280, 3096, 1688, 1632, 11,616, 1595, 1564 and 1386 (NO_2); ^1H NMR (500 MHz, DMSO-d_6): δ 12.29 (s, 1H, Indole-NH (*E*)), 11.98 (s, 1H, Indole-H (*Z*)), 11.91 (s, 1H, CONH (*E*)), 11.53 (s, 1H, CONH (*Z*)), 8.40 (s, 1H, –N=CH–), 7.88 (d, 1H, $J = 3.1$ Hz, Furan H_4 (*Z*)), 7.81 (d, 1H, $J = 3.1$ Hz, Furan H_4 (*E*)), 7.75 (s, 1H, Indole H_3 (*Z*)), 7.69 (d, 1H, $J = 7.5$ Hz, Indole H_7), 7.46 (d, 1H, $J = 7.5$ Hz, Indole H_4), 7.39 (s, 1H, Indole H_3 (*E*)), 7.35 (d, 1H, $J = 3.1$ Hz, Furan H_3 (*Z*)), 7.29 (d, 1H, $J = 3.1$ Hz, Furan H_3 (*E*)), 7.25 (t, 1H, $J = 7.5$ Hz, Indole H_6), 7.09 (t, 1H, $J = 7.5$ Hz, Indole H_5). ^{13}C NMR (125 MHz, DMSO-d_6): δ 157.81 (C=O), 151.80 (C2′ 5-nitrofuran-2-yl (*E*)), 151.28 (C2′ 5-nitrofuran-2-yl (*Z*)), 148.11 (C5′ 5-nitrofuran-2-yl (*E*)), 137.21 (C5′ 5-nitrofuran-2-yl (*Z*)), 137.01 (C7a), 134.65 (C=N), 126.88 (C2), 124.41 (C3a (*Z*)), 124.26 (C3a (*E*)), 121.94 (C6), 121.78, 120.29 (C4 (*Z*)), 120.11 (C4 (*E*)), 118.01 (C5), 115.23 (C4′ 5-nitrofuran-2-yl (*Z*)), 114.68 (C4′ 5-nitrofuran-2-yl (*E*)), 113.79 (C3′ 5-nitrofuran-2-yl), 112.42 (C3), 104.97 (C7 (*Z*)), 104.42 (C7 (*E*)). ESI-Mass *m/z*: 299 $[M + H]^+$, 321 $[M + Na]^+$; Anal. Calcd. for $C_{14}H_{10}N_4O_4$: C, 56.38; H, 3.38; N, 18.79. Found: C, 56.87; H, 3.60; N, 18.40.

*N′-(5-bromo-2-hydroxybenzylidene)-1H-indole-2-carbohydrazide (**2l**)* The derivative **2l** was obtained as an off-white solid by condensation of **B2** with 5-chloro-2-nitrobenzaldehyde in 76 % yield, mp 278–280 °C. IR (KBr) cm^{-1}: 3564 (ν OH), 3355, 3216, 3122, 3058, 1623, 1602, 1565, 1538, 1520. ^1H NMR (500 MHz, DMSO-d_6): δ 12.21 (s, 1H, Indole-NH), 11.85 (s, 1H, CONH), 11.21 (s, 1H, OH), 8.63 (s, 1H, –N=CH–), 7.83 (d, 1H, $J = 2.6$ Hz, –N=CH–C_6H_4, H_6), 7.69 (d, 1H, $J = 8.0$ Hz, Indole H_7), 7.48 (d, 1H, $J = 8.0$ Hz, Indole H_4), 7.44 (dd, 1H, $J = 8.7$, 2.5 Hz, –N=CH–C_6H_4 H_4), 7.35 (s, 1H, Indole H_3), 7.24 (t, 1H, $J = 8.0$ Hz, Indole H_6), 7.08 (t, 1H, $J = 8.0$ Hz, Indole H_5), 6.92 (d, 1H, $J = 8.7$ Hz, –N=CH–C_6H_4, H_3).^{13}C NMR (125 MHz, DMSO-d_6): δ 157.50 (C=O), 156.25 (C2′ 5-bromo-2-hydroxyphenyl), 144.55 (C=N), 136.92 (C7a), 133.43 (C4′ 5-bromo-2-hydroxyphenyl), 130.06 (C2), 129.53 (C6′ 5-bromo-2-hydroxyphenyl), 126.92 (C3a), 124.00 (C6), 121.84 (C4), 121.53 (C2′ 5-bromo-2-hydroxyphenyl), 120.01 (C5), 118.61 (C3′ 5-bromo-2-hydroxyphenyl), 112.40 (C3), 110.48 (C5′

5-bromo-2-hydroxyphenyl), 103.97 (C7). ESI-Mass *m/z*: 358 $[M + H]^+$, 365 $[M + Na]^+$; Anal. Calcd. for $C_{16}H_{12}BrN_{43}O_2$: C, 53.65; H, 3.38; N, 11.73. Found: C, 53.89; H, 3.02; N, 12.02.

N′-(3-methoxybenzylidene)-1H-indole-2-carbohydrazide (**2m**) The derivative **2m** was obtained as an off-white solid by condensation of **B2** with 3-methoxybenzaldehyde in 93 % yield, mp 129–130 °C. IR (KBr) cm^{-1}: 3407–3271, 3061, 2929, 1628), 1610, 1599, 1574, 1529; ^1H NMR (500 MHz, DMSO-d_6): δ 11.89 (s, 1H, Indole-NH), 11.82 (s, 1H, CONH), 8.43 (s, 1H, –N=CH–), 7.69 (d, 1H, $J = 7.5$ Hz, Indole H_7), 7.59 (s, 1H, –N=CH–C_6H_5, H_2), 7.54 (d, 1H, $J = 7.5$ Hz, Indole H_4), 7.48 (d, 1H, $J = 8.0$ Hz, –N=CH–C_6H_5, H_6), 7.37 (t, 1H, $J = 8.0$ Hz, –N=CH–C_6H_5, H_5), 7.33 (s, 1H, Indole H_3), 7.27 (d, 1H, $J = 7.45$ Hz, –N=CH–C_6H_5, H_4), 7.23 (t, 1H, $J = 7.5$ Hz, Indole H_6), 7.08 (t, 1H, $J = 7.5$ Hz, Indole H_5), 2.39 (s, 3H, $–CH_3$). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 157.89 (C=O), 153.50 (C3′ 3-methoxyphenyl), 148.74 (C=N), 146.32 (C7a), 138.07 (C1′ 3-methoxyphenyl), 133.41 (C2), 131.72 (C5′ 3-methoxyphenyl), 127.57 (C3a), 126.18 (C6′ 3-methoxyphenyl), 124.96 (C6), 122.63 (C4), 121.58 (C5), 118.19 (C4′ 3-methoxyphenyl), 114.57 (C2′ 3-methoxyphenyl), 111.35 (C3), 104.05 (C7), 55.76 (O-CH3). ESI-Mass *m/z*: 294 $[M + H]^+$, 315 $[M + Na^+]$; Anal. Calcd. for $C_{17}H_{15}N_3O_2$: C, 69.61; H, 5.15; N, 14.33. Found: C, 69.84; H, 4.93; N, 14.53.

Biological activity evaluation

In vitro platelet aggregation

Platelet aggregation was evaluated by turbidimetric method reported by Born (Born 1962) using APACT 4004 aggregometer. All the synthesized compounds were evaluated for their ability to inhibit platelet aggregation of human platelet-rich plasma (PRP) induced by AA, ADP and collagen as potent aggregation inducers, and using indomethacin and aspirin as standards.

In vitro cytotoxicity assay

To determine the potential cytotoxicity of synthesized derivatives against fibroblast L929, HepG2 (human liver carcinoma), MCF7 (human breast adenocarcinoma) and HeLa (human cervix adenocarcinoma) cell lines, MTT [3-(4,5-dimethylthiazol-2-yl-2,5-tetrazolium bromide)] method was used. The cells were maintained in RPMI1640 medium containing 10 % (v/v) heat-inactivated fetal bovine serum (FBS) supplemented with penicillin (100 U/mL) and streptomycin (100 g/mL) at 37 °C under 5 % CO_2. Cells dispersed at a concentration of 6000 cells/mL were seeded into 96-well plates and allowed to incubate for 24 h. Then, medium was aspirated and various concentrations of test compounds in complete medium were added to each well. After further incubation at 37 °C for 24 h/48 h, the medium was discarded and MTT solution (2 mg/mL, 100 µL) was added to the wells and the mixture was incubated for 3 h at 37 °C. The produced formazan crystals were dissolved in 100 µL of DMSO. Plates were incubated for 20 min at 37 °C, and the optical densities were read at 570 nm with a reference wavelength of 630 nm as background using a spectrophotometer plate reader (Infinite® M200; TECAN, Morrisville, NC, USA). DMSO was used as the solvent of the test compounds, and its final concentration was less than 0.2 %. IC_{50} values were calculated by GraphPad Prism 5.04. All the tests were performed in triplicate (Mashayekhi *et al*., 2013a, b).

Results and discussion

Chemistry

The *N*-acylhydrazones were synthesized through a classic imine formation reaction between indolehydrazides and different aldehydes. The ^1H NMR and ESI-mass data of compounds approved the exact structures. In the 1H NMR spectra of these compounds, the existence of two singlets at 11–12 ppm was assigned to hydrazide NH and indole-NH also. Singlet signal at 8.2–8.8 ppm was assigned to H–C=N. Molecular mass of all the derivatives was detected by electron spray ionization mass spectrometry (ESI–MS) as M + 1 and/or M + 23 and/or M + 39 relating to hydrogen and sodium and potassium adducts of the intact molecules, respectively.

The acylhydrazone structures gave two sets of signals in NMR spectra which their intensities depend on solvent and the pair of signals coalesces on warming the NMR tube. The existence of the carbonyl oxygen atom and the imine nitrogen atom in *N*-acylhydrazones indicates geometrical isomers (*E/Z*). Syakaev *et al*. (2006) also noticed that derivatives of acylhydrazones from aromatic aldehydes showed two rotamers (*cis* and *trans*) due to the N–C(O) bond (Syakaev *et al*. 2006; Unsal-Tan *et al*., 2010).

In our study, the 1H NMR spectra at room temperature for some compounds of **b** and **c** series represented *E/Z* isomerization which was related to C=N double bonds. Right after dissolution of the synthesized compounds in DMSO-d_6, the double signals of $E_{C=N}/Z_{C=N}$ conformers are registered by NMR. The two conformers show two sets of signals at different chemical shifts, and integration of the two sets of signals indicates that the $E_{C=N}$ conformer is the predominant conformer. Based on the earlier report that *N*-acylhydrazones derived from aromatic aldehydes in

solution remained mostly in the *E* form because of the hindered rotation on the imine bond (Palla *et al*., 1986), we considered *E* geometry in our cases.

Anti-platelet aggregation activity

Anti-platelet aggregation activity of the synthesized compounds is shown in Table 1. All derivatives were initially tested at 250 μM concentration, and IC_{50} values were calculated for those with more than 50 % platelet aggregation inhibitory effect.

The inhibition of platelet aggregation induced by collagen, AA and ADP was evaluated for 2 series of acylhydrazone compounds as indole-3-carbaldehyde and indole-2-carbaldehyde derivatives. In general, indole-3-carbaldehyde derivatives demonstrated higher activity against platelet aggregation induced by collagen than the other group. Compounds **3e** and **3b** exhibited the highest activities against the platelet aggregation induced by collagen with IC_{50} values of 12.7 μM and 13.3 μM, respectively, which are comparable to that of Aspirin (9.7 μM), a well-known non-selective cyclooxygenase inhibitor. Nevertheless, compounds in **c** series showed higher activities against the platelet aggregation induced by arachidonic acid. In this group, **2h** with IC_{50} value of 51.88 μM and **2i** with IC_{50} of 44.38 μM were the most potent compounds among indole-2-carbaldehyde derivatives which are comparable with aspirin (30 ± 2.6). Compound **3k** exhibited promising inhibitory effect against platelet aggregation agonist of ADP which shows considerable activity in comparison with indomethacin (42 %) and aspirin (21 %) as standards.

Mirfazli et al. have reported a group of *N*-acylhydrazones of indole ring, among which the most active compound has been compound **2a** with the IC_{50} value of 94 ± 1.9 μM against the aggregation induced by arachidonic acid (AA). In the present study, a larger group of *N*-acylhydrazone derivatives of indole has been synthesized with more diverse structures. Compound **2i** with a 2,3-dihydroxyphenyl moiety has shown a twofold activity against AA-induced aggregation when compared with compound **2a**. Furthermore, in the new series, some of the compounds such as 3e, 3b and 3d have shown great activities against collagen-induced platelet aggregation (Table 1).

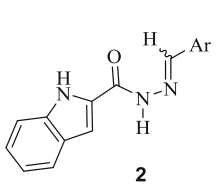
Cytotoxicity assay

The synthesized derivatives were evaluated for their cytotoxicity against three cancer cell lines and L929 as a non-cancer one. As evident from the activity data listed in Table 2, none of the compounds showed a significant cytotoxic effect either against the normal cell L929 or cancerous cell lines.

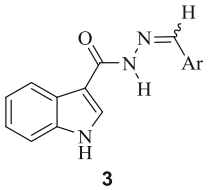
QSAR study

In order to explore the important physicochemical and structural parameters responsible for the modulation of the anti-platelet aggregation activities of the synthesized compounds, quantitative structure–activity relationship (QSAR) analysis with different types of molecular descriptors was carried out. Various physicochemical descriptors as independent variables (e.g., topological indices, hydrophobic and steric parameters) were used in QSAR modeling studies to find the impacts of different structural properties on the biological activity of the synthesized (except **3c**, **3p** and **3q** which did not dissolved). The IC_{50} values were transformed into pIC_{50} values (i.e., $-\log IC_{50}$) and determined as a dependent variable in the QSAR study. In the present study, a data set of the twenty-eight structures of titled compounds was subjected to stepwise linear regression analysis for model generation. During this analysis, it was observed that the response value of one compound only in first model (AA data set) was outside the limits of response values of other synthesized compounds. Thus, the compound was considered as outliers and was not involved in the data set for QSAR model generation. Since the calculated values of some electronic descriptors depend on the three-dimensional molecular geometry, the optimum 3-D geometry of the molecules was obtained by Hyperchem software (version 7, Hypercube Inc, USA), using AM1 semi-empirical method. Dragon software (Todeschini http://www.disat.unimib.it/vhm/) was employed to obtain constitutional, functional, geometrical and topological descriptors of the synthesized compounds **2** and **3**. Meanwhile, some electronic descriptors such as frontier molecular orbital (HOMO, LUMO), dipole moment (MDP) and partial charges were calculated by the Hyperchem software. Activity data were converted to logarithmic scale (i.e., pIC_{50} for platelet aggregation induced by AA, collagen and ADP). For each set of descriptors, the best multi-linear regression equation was obtained by multiple linear regression (MLR) methods subroutine of SPSS software (SPSS Inc., version 18). The correlation coefficient (R^2), standard error of regression (SE), root-mean-square error (RMS) and significant level (*p* value) were employed to judge the validity of regression equation. Correlation analysis was done to indicate the co-linear descriptors, since colinearity declines the efficiency of the MLR-based QSAR equation. Therefore, the descriptors that correlated with each other and with activity data were determined and the highest correlated descriptor among the co-linear descriptors was hold and the others were removed (Aggarwal *et al*., 2011; Firoozpour *et al*., 2012; Nakhjiri *et al*., 2012; Singh *et al*., 2010). The calculated

Table 2 Cytotoxic activity of the synthesized compounds (**2a–m**) and (**3a–r**)



2



3

Compounds	Anti-cancer activity, IC_{50} (μM)			
	Non-cancer cell line	Cancer cell lines		
	L929	HeLa	HepG2	MCF7
3a	301.23	249.53	228.57	263.14
3b	265.34	278.89	253.78	248.78
3c	≥500	291.32	230.34	201.05
3d	≥500	276.90	234.78	288.90
3e	165.45	148.89	129.90	133.33
3f	277.98	180.96	367.76	259.55
3g	222.77	159.11	239.91	199.23
3h	197.74	182.57	≥500	166.85
3i	≥500	280.90	197.44	≥500
3j	≥500	≥500	159.90	172.57
3k	277.90	250.67	190.98	≥500
3l	384,25	359.90	361.78	247.90
3m	≥500	≥500	≥500	≥500
3n	169.45	160.13	167.89	194.12
3o	≥500	≥500	≥500	≥500
3p	200.35	≥500	378.90	356.01
3q	189.21	323.23	291.35	301.40
3r	≥500	401.21	398.32	320.46
2a	≥500	160.90	133.89	≥500
2b	358.11	261.88	222.67	193.89
2c	≥500	272.25	188.98	248.67
2d	373.50	291.44	≥500	≥500
2e	≥500	≥500	≥500	298.89
2f	144.79	209.71	188.91	167.23
2g	291.29	198.99	323.67	262.10
2h	≥500	320.55	290.45	≥500
2i	≥500	167.23	203.45	391.89
2j	390.43	≥500	233.80	≥500
2k	250.04	302.44	299.80	≥500
2l	130.45	221.67	201.45	214.01
2m	≥500	201.34	391.45	268.28

descriptors in separated equation related to different inducers (AA, collagen and ADP) were shown in data matrices for retained descriptors in Table 3 for Eqs. (1), (2) and (3), respectively. Finally, we selected similar molecules reported in pervious study as external prediction set (Mirfazli *et al.*, 2014). The experimental and predicted activity was shown in Table 4. According to predicted activity, Eqs. (1) and (2) represented good predictive ability; however, Eq. (3) did not show appropriate prediction for selected compounds.

In this study, we used the pIC_{50} of indole acylhydrazone derivatives against AA-induced platelet aggregation as the dependent variable, and Eq. 1 was obtained from the pool of calculated descriptors.

$$\begin{aligned} pIC_{50} = {} & 2.93\,(\pm 1.842) - 0.491\,(\pm 0.064)\,\mathrm{X2sol} \\ & + 0.199\,(\pm 0.042)\,\mathrm{RDF135u} \\ & + 0.002\,(\pm 0.001)\mathrm{TIE} - 0.088\,(\pm 0.027)\,\mathrm{RDF120u} \\ & + 15.128\,(\pm 5.863)\,\mathrm{PW3} \\ n = 27 \quad & R^2 = 0.791 \quad \mathrm{Se} = 0.191 \\ F = 15.93 \quad & R^2_{cv} = 0.67 \quad \mathrm{RMS_{cv}} = 0.218 \\ \mathrm{PE} = {} & 0.018 \end{aligned} \tag{1}$$

Here and thereafter, '*n*' is the number of data points, *R*—correlation coefficient, Se—standard error of the estimate, *F*—Fischer statistic, PE—probable error of the coefficient of correlation.

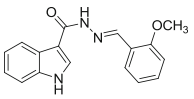
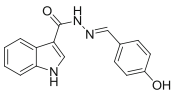
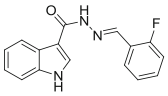
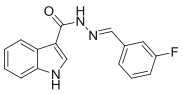
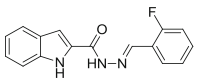
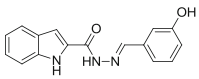
As seen in Eq. (1), this model shows good statistical quality ($R^2 = 0.791$, $\mathrm{Se} = 0.191$, $F = 15.93$) that contains: solvation connectivity index of order 2 (X2sol), Radial Distribution Function-135 unweighted (RDF135u), E-state topological parameter (TIE), Radial Distribution Function-120 unweighted (RDF120u) and path/walk 3-Randic shape index (PW3).

Molecular topology as numerical quantifiers is sensitive to bonding pattern, content of heteroatom (complexity of atomic neighborhoods) and symmetry (Lather and Madan, 2005) (Kumar *et al*., 2014; Rathod *et al*., 2012). In this case, topological index, PW3, was the most dominant parameter in describing the anti-platelet activity of the synthesized compounds against the aggregation induced by AA and a positive correlation was observed. The molecular path/walk indices are specified as the ratio between the atomic path count and the atomic walk count of the same length. Whereas the number of paths in a molecule is bounded and determined by the molecule's diameter, the number of walks is unbounded. However, being interested only in quotients, the walk count is terminated when it exceeds the maximum allowed length of the corresponding path. The average sum of atomic path/walk indices of equal length and its count ratio is independent of molecular size. Therefore, PW3 can be considered as shape descriptor and is related to transport phenomena and interaction capabilities between ligands and receptors (Vahdani and Bayat, 2011; Todeschini and Consonni, 2000). As is apparent in AA model equation, the PW3-positive sign means that increasing the PW3 of molecules leads to decreasing in IC_{50} values.

Table 3 Correlation coefficient (R^2) matrix for some of descriptors used in this study

	X2sol	RDF135u	TIE	RDF120u	PW3
Table Eq. (1): Anti-platelet aggregation activity induced by AA data set					
X2sol	1	0.204	0.183	0.146	0.361
RDF135u		1	0.071	0.582	0.320
TIE			1	0.055	0.001
RDF120u				1	0.521
PW3					1
	LP1	PW4	RDF145u	PJI2	RDF100u
Table Eq. (2) Anti-platelet aggregation activity induced by collagen data set					
LP1	1	0.691	0.251	−0.018	−0.102
PW4		1	−0.080	−0.068	0.284
RDF145u			1	−0.070	−0.236
PJI2				1	−0.120
RDF100u					1
	RDF100v	RDF065u	nR06	RDF115u	GATS6v
Table Eq. (3) Anti-platelet aggregation activity induced by ADP data set					
RDF100v	1	0.701	0.209	0.111	0.256
RDF065u		1	0.274	0.233	0.165
nR06			1	0.343	0.063
RDF115u				1	−0.518
GATS6v					1

Table 4 Experimental and predicted activity of external prediction set

Compounds	AA		Collagen	
	Experimental	Predicted	Experimental	Predicted
	3.50	3.83	3.91	4.29
	3.53	3.73	3.72	4.46
	3.74	3.55	3.91	4.53
	3.73	3.66	4.67	4.50
	3.54	3.88	3.14	3.48
	3.69	3.48	3.72	3.63

Equation (2) describes the most reliable model for inhibitory effect of the synthesized compound in platelet aggregation induced by collagen as below:

$$\begin{aligned} \mathrm{pIC_{50}} &= -207.85\,(\pm 18.126) + 90.871\,(\pm 8.02)\,\mathrm{LP1} \\ &\quad -52.043\,(\pm 8.85)\,\mathrm{PW4} \\ &\quad + 0.625\,(\pm 0.161)\,\mathrm{RDF145m} + 1.697\,(\pm 0.631)\,\mathrm{PJI_2} \\ &\quad + 0.050\,(\pm 0.019)\,\mathrm{RDF100u} \\ n &= 28 \quad R^2 = 0.883 \quad \mathrm{Se} = 0.238 \quad F = 33.316 \\ R^2_{\mathrm{cv}} &= 0.807 \quad \mathrm{RMS_{cv}} = 0.277 \quad \mathrm{PE} = 0.014 \end{aligned} \tag{2}$$

This equation has high statistical quality ($R^2 = 0.883$, Se = 0.238, $F = 33.316$) which has Lovasz–Pelikan index (leading eigenvalue) (LP1), path/walk 4-Randic shape index (PW4), Radial Distribution Function-145 unweighted (RDF145u), 2D Petitjean shape index (PJI2) and Radial Distribution Function-100 unweighted (RDF100u) as important parameters in the Equation.

LP1 is the Lovasz–Pelikan index of leading eigenvalue and is a topological descriptor. It has been suggested as an index of molecular branching, the smallest values corresponding to chain graphs and the highest to the most branched graphs. In Eq. (2), the LP1 contribution has a positive sign, which indicates that the IC_{50} is inversely related to this descriptor; therefore, increasing the branching of molecules leads to a decrease in their IC_{50} (Du *et al.*, 2011). Also in this model, PW4 is another descriptor which can show significant effect in IC_{50} value, but, with negative sign, it has reverse effect in IC_{50} values in collagen-induced platelet aggregation equation.

For Eq. (3), the percent of inhibition for platelet aggregation induced by ADP of the compounds as dependent variable was used and Eq. (3) as ADP model was determined.

$$\begin{aligned} \mathrm{Log}\,(p/100 - p) &= -0.591\,(\pm 0.783) - 0.863\,(\pm 0.105) \\ &\quad \mathrm{RDF100v} + 0.331\,(\pm 0.048)\,\mathrm{RDF065u} \\ &\quad + 1.894\,(\pm 0.367)\,\mathrm{nR06} - 0.299\,(\pm 0.065)\,\mathrm{RDF115u} \\ &\quad - 1.240\,(\pm 0.310)\,\mathrm{GATS6v} \\ n &= 28 \quad R^2 = 0.838 \quad \mathrm{Se} = 0.534 \quad F = 22.781 \\ R^2_{\mathrm{cv}} &= 0.686 \quad \mathrm{RMS_{cv}} = 0.672 \quad \mathrm{PE} = 0.020 \end{aligned} \tag{3}$$

Third equation, Eq. (3), with Radial Distribution Function-100 weighted by van der Waals volume (RDF100v), Radial Distribution Function-065 unweighted (RDF065u), number of 6-membered rings (nR06), Radial Distribution Function-115 unweighted (RDF115u) and Geary autocorrelation of lag 6 weighted by van der Waals volume (GATS6v) as ADP model descriptors defines best statistical quality with

$R^2 = 0.838$, Se = 0.534, $F = 22.781$. Ring descriptor (nR06) as a constitutional descriptor has detrimental effects on percent inhibition activity of the compounds and GATS6v with negative sign determines that decreasing the GATS6v (van der Waals volume) leads to increase in IC_{50} values.

The correlation between the experimental and predicted activities for all of 28 compounds is represented graphically in Fig. 3a, b, c for AA, collagen and ADP model, respectively.

Equations (1) and (2) illustrate that spatial and topological descriptors have important role in anti-platelet aggregation activity of the synthesized compounds. The similarity of descriptors in Eqs. (1) and (2) represents that structures inhibit platelet aggregation through approximately same mechanism. In Eq. (3), the autocorrelation vectors represent the degree of similarity between molecules. Therefore, anti-platelet aggregation activity of these compounds is mainly dependent on molecular shape of the structure. The other important descriptors that presented in all models were RDF descriptors. RDF (radial distribution function) descriptors as a similar parameter were emerged in all equations for this study data set, which are based on the distances distribution in the geometrical representation of a molecule and constitute a radial distribution function code. Additionally, the RDF descriptors can be restricted to specific atom types or distance ranges to represent specific information in a certain three-dimensional structure space, e.g., to describe steric hindrance or structure/activity properties of a molecule, and also these descriptors display valuable information, for example, about bond distances, ring types, planar and non-planar systems and atom types. Topological indices were other important descriptors. These indices help to differentiate the molecules according mostly to their size, degree of branching, flexibility and overall shape (Puzyn *et al.*, 2010).

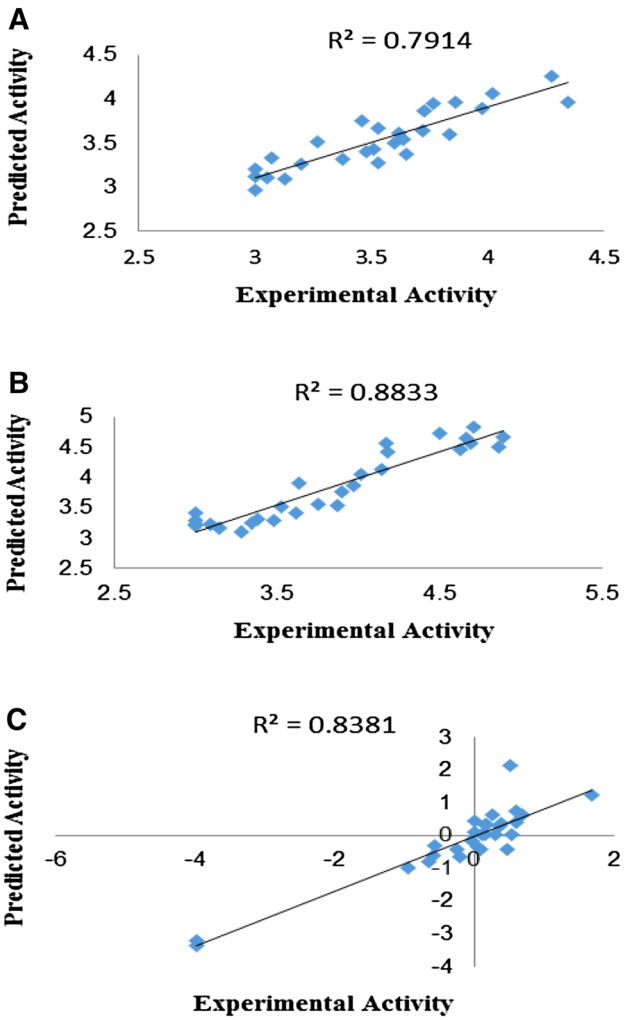


Fig. 3 Plots of cross-validated predicted values of anti-platelet aggregation activity by MLR against the experimental values for different aggregation inducers: (**3A**) AA, (**3B**) collagen and (**3C**) ADP

Conclusion

We have designed and synthesized thirty-one indole *N*-acylhydrazone derivatives **2**(**a**–**m**) and **3**(**a**–**r**). It was found 3-indole substituted derivatives are more active than other compounds in platelet aggregation inhibitory effect.

In addition, comparing the activity of compounds against platelet aggregation induced by AA shows that compounds **2**(**a**–**m**) have less molecular diameters than the compounds **3**(**a**–**r**) and increasing in path/walk indices decreases IC_{50} values according to the Equation. However, in collagen model, LP1 with positive sign and also PW4 as path/walk index with negative sign, demonstrating the branching and increasing in molecular diameter (shape decides its nature of binding in the active site), result in higher anti-platelet aggregation activity in 3-indole derivatives.

In general, *N*-acylhydrazones showed different activity profiles against the platelet aggregations induced by ADP, AA and collagen. Furthermore, the QSAR study demonstrated that different descriptors such as constitutional, topological and geometrical play important role in anti-platelet aggregation activity of the synthesized compounds against mentioned inducers.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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